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ENDOCRINOLOGY

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INDUCTION OF OVULATION IN THE DIESTROUS MOUSE BY GONADOTROPINS

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THE INDUCTION of ovulation in adult mice by a single injection of chorionic gonadotropin was reported by Burdick and Whitney (1941). Later Burdick et al (1943) developed this into an assay method. Using adult diestrous mice, and administering the hormone in a single subcutaneous injection, they found that 0.75 I.U. of chorionic gonadotropin induced ovulation in some cases within 24 hours, while 1 I.U produced ovulation in 100 per cent of the mice. They proposed a unit based on ovulation in 50 per cent of the animals. Although they did not test either pregnant mare's serum or pituitary extracts in adult diestrous mice, they found that in immature mice, chorionic gonadotropin was 2 to 4 times as effective as pregnant mare's serum when both were standardized in international units.

MATERIAL AND METHOD

We were interested in the method as an assay and also as an additional means of comparing gonadotropins from various sources. We were further interested in determining whether the ovulation was induced by follicle stimulating hormone (FSH), luteinizing hormone (LH), or a combination of these. For comparative purposes we used two commercial preparations of chorionic gonadotropin (PU), two commercial preparations of pregnant mare's serum (PMS) and a number of pituitary extracts, some fractionated and some containing both FSH and LH. In order to establish a basis of comparison, all preparations were assayed according to our standard method as follows: The material is administered in 6 equal injections, twice daily for three days, to immature female rats of the Sprague-Dawley strain starting when the animals are 23–24 days of age. The animals are sacrificed 96 hours after the first injection and the uteri are weighed after first expressing any fluid from the uterine cavity. At least 6 rats are used at every dose level.

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The rat unit (R.U.) is defined as the amount required to produce an average increase in uterine weight amounting to 100 per cent.

The seven hundred albino mice used in these experiments were obtained from two sources but no differences in responses were noted. They were all adults, weighing on an average 20 to 25 grams at autopsy. Daily vaginal smears were taken on all mice used in these experiments and, in general, the animals were injected on the first day of diestrus. However, in a considerable number of mice, no estrous cycle could be established. Some of these animals were also used and in all cases the smear on the day of autopsy showed that the animals remained in diestrus. Data on some of these animals are included in table 1. No differences were observed in the sensitivity of the

TABLE 1. COMPARISON OF THE EFFECTIVENESS OF VARIOUS GONADOTROPINS IN CYCLING AND NON-CYCLING DIESTROUS MICE

Preparation	Dose	Injected on first day of diestrus		Injected after showing no estrus for at least 5 weeks	
		Number injected	Number ovulating	Number injected	Number ovulating
None		32	0	14	0
PMS-1	5 I.U.	3	3	2	2
	2 I.U.	8	4	2	0
231	0.5 mg.	3	3	2	2
	0.25	1	1	4	4
	0.1	1	0	4	4
	0.05	5	4	5	5
	0.025	5	2	5	0
112	0.1 mg.	5	4	5	4
	0.05	5	4	5	2
	0.025	5	0	5	4
331	0.1 mg.	2	2	3	2
	0.04	5	1	5	1
	0.02	5	1	5	0

mice showing cyclic estrus as compared with the non-cyclic animals. Therefore, all of the results are combined in this paper. The hormone was administered in a single subcutaneous injection, usually in a volume of 0.25 ml. After 24 hours, the animals were sacrificed and the oviducts examined for swellings under the dissecting microscope. In all cases, the oviducts were serially sectioned and the ova counted. The counts reported here are average numbers of ova per mouse ovulating.

RESULTS

Two commercial preparations of chorionic gonadotropin were used. These were dry powders and were diluted so as to contain a listed 100 I.U. per ml. By our assay PU-1 (Korotrin) was found to contain 100 R.U. per ml. while PU-2 (Pregnyl) assayed 300. Lack of material prevented us from obtaining a complete assay on PU-1. Table 2 shows that about 8 R.U. of PU-2 are required to produce ovulation in 50% of the cases. In our hands chorionic gonadotropin was much less effective than the results of Burdick showed.

Two preparations of pregnant mare serum were also used. These

were also dry powders which we diluted to contain a listed 100 I.U. per ml. We obtained the following assays:

PMS-1 (Anteron) 200 R.U. per ml.
PMS-2 (Gonadogen) 170 R.U. per ml.

Table 2 shows that from 2 to 5 R.U. of PMS will cause ovulation in 50% of the mice.

From the data reported by Burdick, we can calculate that from 1.5 to 8 R.U. of PMS are required to produce ovulation in 50% of the mice. Our results are in good agreement with these figures.

The pituitary extracts tested will be grouped for comparison. The

TABLE 2. OVULATION PRODUCED BY PU AND PMS

Preparation	Dose I.U. Stated	Dose R.U. Found	No. of Mice	Percent Ovulating	Average No. of Ova
PU-1	2.0	2.0	4	0	—
	1.	1.	5	20	7
PU-2	5.	15.	10	90	9.7
	2.	6.	10	30	5.4
	1.	3.	10	10	3.0
	0.5	1.5	10	0	—
PMS-1	5.	10.	5	100	6.8
	2.	4.	3	100	15.
	1.	2.	10	40	3.
	0.4	0.8	2	0	—
	0.2	0.4	1	0	—
PMS-2	4.	6.8	10	80	8.1
	2.	3.4	20	0	—
	1.	1.7	9	0	—

first group, shown in table 3, includes those prepared by the method we use in manufacture of Gonadophysin, but prepared from glands of various species. These must be listed as unfractionated extracts since they produce both follicle stimulation and luteinization. Except where otherwise noted, all pituitary extracts were prepared in our laboratory.

131. Sheep pituitary. 12 R.U. per mg. Ovulating dose 0.36 R.U.

331. Horse pituitary. 38 R.U. per mg. Ovulating dose 2.25 R.U.

231. Pig pituitary. 3.5 R.U. per mg. Ovulating dose 0.12 R.U.

Although these were all prepared by the same method, we see from table 3 that there is a marked difference in their relative effectiveness in immature rats and adult mice. A brief series was run on some acetone dehydrated human pituitaries, 431. This preparation assays 10 R.U. These results are included in table 3.

The effects of various procedures on pituitaries from a common source were also investigated. In table 3, we illustrated an extract from sheep pituitary which has both FSH and LH activity. In table 4 we list a number of extracts, prepared by methods recommended for FSH while in table 5 we have LH and prolactin preparations. All were prepared from sheep pituitary glands.

The first of these, 111 was the initial extract by the method of Fevold et al. (1940). No repurification was attempted and from other tests it obviously contains considerable LH. The ovulation dose, 0.24 R.U. is similar to that reported for the unfractionated extracts

TABLE 3. EFFECTIVENESS OF EXTRACTS OF PITUITARIES FROM VARIOUS SPECIES

No.	Species	Dose—mg.	Dose—R.U.	No. of mice	Percent ovulating	Average No. of ova
131	Sheep	0.84	10.	5	80	3.0
		0.42	5.	5	100	6.4
		0.168	2.	5	100	6.4
		0.084	1.	5	100	6.6
		0.042	0.5	10	90	6.1
		0.021	0.25	10	10	5.0
331	Horse	0.1	3.8	5	80	6.3
		0.08	3.0	10	70	6.4
		0.04	1.5	9	22	6.5
		0.02	0.76	10	10	10.
231	Pig	0.5	1.72	5	100	4.6
		0.25	0.86	5	100	6.4
		0.1	0.35	5	80	4.3
		0.05	0.172	10	90	5.7
		0.025	0.086	10	20	4.5
431	Human	0.5	5.	10	90	9.7
		0.25	2.5	10	30	2.3
		0.125	1.25	5	20	1.
		0.0625	0.625	5	0	

TABLE 4. EFFECTIVENESS OF VARIOUS FSH PREPARATIONS

Preparation	Dose—mg.	Dose—R.U.	No. of Mice	Percent Ovulating	Average No. of Ova
111	2.0	1.14	4	75	5.3
	1.	0.57	10	90	5.0
	0.5	0.286	10	70	7.0
	0.25	0.143	9	0	
112	0.2	1.56	10	80	5.5
	0.1	0.78	10	80	5.1
	0.05	0.39	10	60	4.3
	0.025	0.195	10	40	4.3
	0.0125	0.098	5	0	
113	0.05	0.44	10	50	5.4
	0.025	0.22	10	70	4.6
	0.0125	0.11	10	70	4.9
	0.00625	0.055	10	0	
114	0.125	1.2	10	90	4.2
	0.0625	0.6	10	60	6.0
	0.03125	0.3	10	40	1.5
	0.01563	0.15	10	20	3.0

but slightly more active. 112 and 113[1] were prepared by the method of Pincus, the latter in his laboratory. 112 has about the same activity as 111 while 113 is somewhat more active.

[1] Furnished through the courtesy of Dr. Pincus, Worcester Foundation for Experimental Biology, Shrewsbury, Massachusetts.

114 was prepared by a method involving removal of LH with picric acid. In a limited series of hypophysectomized rats, this produced relatively few corpora lutea and only at high doses. In mice we see that it is slightly less effective than the unfractionated extract.

As will be shown later, the differences shown by these 5 extracts from sheep pituitary are relatively small when compared to the differences exhibited by all the gonadotropins tested.

The LH preparations shown in table 5 have little effect when administered alone to immature female rats. However, their LH activity has been demonstrated by their effect on immature male rats.

121 was prepared by Fevold's method but not repurified. The rat

TABLE 5. EFFECT OF LH AND PROLACTIN ON DIESTROUS MICE

Preparation	Dose	No. of mice	Percent ovulating	Average No. of ova
121	8 mg.	10	70	4.5
	4	10	70	4.6
	2	10	20	5.0
122	1.0 mg.	10	30	7.0
	0.5	10	60	5.7
	0.2	10	40	1.
	0.1	10	10	1.
	0.04	6	17	7.
	0.02	10	0	—
	0.01	4	0	—
141	1.0 mg.	5	0	—
	0.2	4	0	—

unit is 32 mg. However 8 mg. produced a 50% increase in seminal vesicle weight in the male rat. 3 mg. is the ovulating dose.

122[1]—Prepared for us by Dr. Pincus. The rat unit is more than 1 mg. but this dose produced a 90% increase in the weight of the seminal vesicles of immature male rats. About $\frac{1}{3}$ this amount produced ovulation in mice.

141[1] is a prolactin preparation containing 20 I.U. per mg. It failed to produce ovulation in mice.

DISCUSSION

Table 6 presents a summary of all the gonadotropins tested. It is significant that all sheep preparations produced ovulation in a dose of 0.09 to 0.42 R.U. while all other preparations tested except pig pituitary, required at least 5 times this much. While the various sheep pituitary extracts showed relatively little variation in the dose, in R.U., to produce ovulation in mice, some differences in the effects of these preparations were noticeable. The unfractionated extract produced ovulation in 100% of the mice over quite a wide range of doses. With FSH we rarely obtained more than 70 or 80%. With the LH preparation at least in one case, the effectiveness dropped markedly past an optimal dosage. We may explain this latter effect

as being due to premature luteinization of the follicle, before ovulation can take place.

It is remarkable that the most potent sheep pituitary extract is some ninety times as effective as the pregnancy urine extract tested, in inducing ovulation in mice when both extracts are standardized in rat units. The relative effectiveness of pituitary extracts in inducing ovulation has often been reported. Saunders and Cole (1938) using rats and rabbits found that it took 16 R.U. of PMS to induce ovulation in the estrous rabbit, but 0.25 R.U. of a sheep pituitary FSH or 0.0125 R.U. of a sheep pituitary LH would produce this effect.

TABLE 6. RELATIVE EFFECTIVENESS OF VARIOUS GONADOTROPINS

Preparation		Dose to ovulate 50% of mice
113	Sheep FSH	0.09 R.U.
231	Pig pituitary UNF	0.115
111	Sheep FSH (crude)	0.235
112	Sheep FSH	0.275
131	Sheep pituitary UNF	0.355
114	Sheep pituitary FSH	0.42
331	Horse pituitary UNF	2.25
PMS-1	Pregnant mare serum	2.25
431	Human pituitary—dried UNF	3.15
PMS-2	Pregnant mare serum	5.25
PU-2	Pregnancy urine	8.10
121	Sheep pituitary LH	0.125
122	Sheep pituitary LH	<0.33

The FSH and LH preparations used in these experiments were relatively crude, and the rat unit used was defined as the amount required to produce a definite effect on the immature rat ovary. Rowlands and Williams (1943) using a somewhat larger rat unit, i.e., the amount required to produce 30 mg. ovaries, found the following numbers of R.U. were required to produce ovulation in 50 per cent of the adult estrous rabbits:

horse pituitary	0.18
gelding pituitary	0.28
International Standard PMS	1.2

They also found horse pituitary more effective than PMS in producing ovulation in intact and hypophysectomized immature rats. Weisman and Coates (1943) using the African clawed toad found that 25 R.U. sheep pituitary, Gonadophysin, produced ovulation but that 30 Cartland-Nelson units PMS, Gonadogen, (about 400 R.U. by our tests) or 100 R.U. PU, Antuitrin S, (about 200 R.U. by our tests) were required.

We had hoped to determine by these experiments whether it was the FSH or LH factor, or a definite combination of these, which is

responsible for ovulation. Since these two factors cannot be properly compared by any test, we cannot say that either is more effective in inducing ovulation. However, it is evident that either factor will induce ovulation in the diestrous mouse in a fraction of the dosage required to produce either a significant increase in uterine weight or an increase in weight of seminal vesicles of the immature rat.

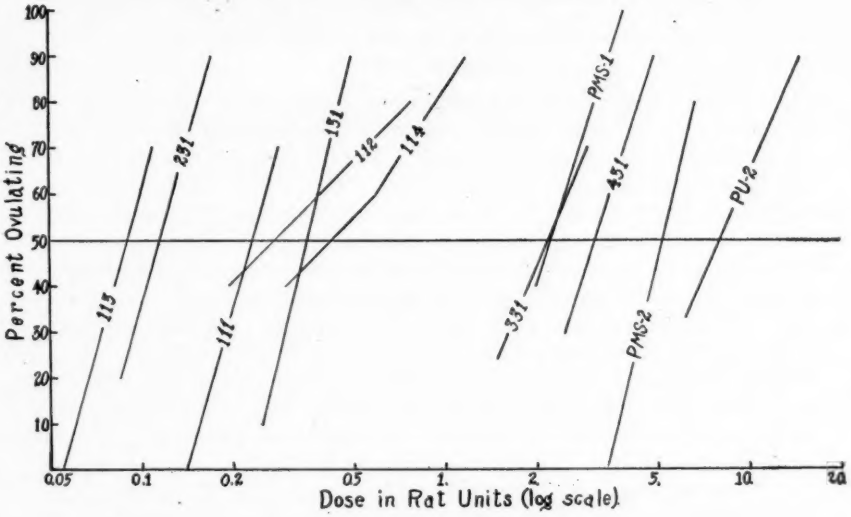

FIG. 1. Comparative potency of various gonadotropins in immature rats and mature diestrous mice.

Figure 1 is a graphic representation of the relative effectiveness of the various gonadotropins. The dosage is plotted on a logarithmic scale.

SUMMARY

A number of gonadotropic preparations were compared on the basis of their relative potency in causing uterine weight increases in immature rats, and in inducing ovulation in the diestrous mouse. While 8 uterine R.U. of PU or 2 to 5 R.U. of PMS were required to induce ovulation in 50% of the mice, smaller amounts of sheep or pig pituitary extracts produced the same effect. These ranged from 0.42 R.U. to as little as 0.09 R.U. of sheep pituitary extract. The effectiveness of pituitary extracts in inducing ovulation in the mouse and in other species was discussed.

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REFERENCES

BURDICK, H. O., AND R. WHITNEY: *Am. J. Physiol.* 132: 405. 1941.
BURDICK, H. O., HUBER WATSON, VINCENT CIAMPA AND THOMAS CIAMPA: *Endocrinology* 33: 1. 1943.
FEVOLD, H. L., MILTON LEE, F. L. HISAW AND E. J. COHN: *Endocrinology* 26: 999. 1940.
ROWLANDS, I. W., AND P. C. WILLIAMS: *J. Endocrinol.* 3: 310. 1943.
SAUNDERS, F. J., AND H. H. COLE: *Endocrinology* 23: 302. 1938.
WEISMAN, ABNER I., AND CHRISTOPHER W. COATES: *J. Lab. & Clin. Med.* 28: 1631. 1943.

THE ACTION OF HUMAN PROSTATE SLICES ON α-ESTRADIOL AND ESTRONE[1]

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DURING the course of an investigation into the metabolism of estrogens in the prostate gland, it was observed that slices of normal human prostate, suspended in a Ringer-phosphate-glucose medium containing either estrone or α-estradiol, were capable of causing the disappearance of a large proportion of the estrogen from the medium. In attempting to account for the fate of estrone, we found that the amount of the estrogen which disappeared from the medium could be completely recovered from the slices by extraction of the latter with ethanol and ether. There was therefore no *net* destruction of estrone by the prostate slices. The removal of the estrogen from the medium and its retention by the slices appears *not* to be a specific metabolic process, since slices of prostate or of rat liver previously heated to 100° C, possessing no respiratory activity, were equally capable of causing the removal of estrone or α-estradiol from the medium. Normal untreated, actively respiring slices of rat liver caused rapid disappearance of α-estradiol from the medium, but in this case no estrogen could be recovered from the slices by extraction procedures. The removal of estrogen from the medium by normal rat liver slices therefore represents a metabolic destruction of the estrogen, which has already been studied extensively *in vitro* by several investigators, including Zondek and Sklow, Twombly and Taylor, and Singher et al. The removal of estrogen by prostate slices or heat-inactivated slices apparently represents a non-specific, non-metabolic adsorption of the estrogen.

With the increasing interest in the intermediary metabolism of estrogens, it is expected that the *in vitro* technique will be more extensively used in investigations into the sites and mechanisms of estrogen destruction and formation. Since the non-specific adsorption of estrone and α-estradiol by tissue slices described here is an effect of considerable magnitude, we wish to present some details concerning the standardization of the *in vitro* technique for investigations of this type and some experimental data indicating the magnitude of the adsorption effect.

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EXPERIMENTAL

Phosphate-saline solutions containing estrone or α-estradiol in concentrations from 0.10 to 2.0 micrograms per ml. were prepared by the following method: For a phosphate-saline buffer containing 1.0 microgram per ml., a stock solution of hormone in phosphate buffer was first prepared by weighing and transferring 2.0 mg. of hormone into a 250 ml. beaker. The hormone was brought into solution by the addition of 3.0 ml. of 20% NaOH and warming on a hot plate. Not all the material dissolved immediately, but on gradual addition of water and constant stirring with a glass rod, maintaining the temperature at 60° or higher, all the estrogen dissolved. A total of 180 ml. of water was finally added. To the clear, hot solution was added 2.76 g. of solid $NaH_2PO_4 \cdot H_2O$ gradually with stirring, yielding a clear solution. This was allowed to cool to 40° C. and was then filtered into a 200 ml. volumetric flask and made to volume with water. The resulting solution consists of 0.10 M phosphate buffer, pH 7.2, containing 10 micrograms of hormone per ml. This solution was stable for at least 24 hours at room temperature, but the hormone gradually precipitated from the solution at refrigerator temperatures. The directions given above for the stock solution gives a final hormone concentration in the completed buffer described below of 1 microgram per ml. By varying the amount of estrogen in the stock solution from 1 to 20 micrograms per ml., leaving phosphate concentration constant, a complete phosphate-saline buffer medium containing anywhere between 0.1 and 2.0 micrograms per ml. can be prepared. The stock solution was usually used immediately to prepare the complete buffer medium which consisted of 12.0 ml. of the stock hormone-phosphate solution, 20 ml. 0.775 M NaCl, 0.80 ml. 0.775 M KCl, 0.20 ml. 0.775 M KH_2PO_4, 0.20 ml. 0.775 M $MgSO_4$, 1.2 ml. 10% glucose and water to make 120 ml.

This medium was stable for 2 or 3 days at refrigerator temperatures without formation of turbidity. Calcium salts (usually present in buffers used as media for surviving slices) caused immediate precipitation of hormone from the medium and were therefore omitted. The buffer was usually used within a few hours after preparation. It is felt that the method for preparing aqueous hormone solutions described above is more satisfactory than methods in which an acetone or alcohol solution of hormone is diluted with aqueous buffer.

Slices of the tissues used were collected in Petri dishes containing oxygenated buffer of the same composition as described above with hormone omitted. The slices were freed of medium by blotting on filter paper, weighed to 0.1 mg. on cellophane strips on a torsion balance, and transferred to standard Warburg vessels containing the appropriate media and alkali and filter paper in the center well. The vessels were gassed with oxygen and brought to temperature equilibrium in the bath at 38° C. Manometric readings of oxygen uptake were taken at 15 minute intervals.

At the conclusion of the incubation period, the slices were removed from the vessels, drained on filter paper, immersed in 10 ml. hot ethanol, and held for extraction and assay. The medium of each flask was transferred to a 15 ml. graduated centrifuge tube and the volume noted. The tubes were immediately placed in a boiling water bath for 15 minutes and cooled. The volume of each tube was restored with water. The media were mixed and centrifuged at high speed for 15 minutes. Samples of the clear supernatants were withdrawn for dilution with saline and subsequent bioassay. The rest of the supernatant was drained from the tubes without disturbing the precipitate of heat-coagulated protein. This precipitate was covered with 10 ml. of hot ethanol and held for extraction and analysis.

The slices and precipitates were then extracted separately under reflux with, successively, 20 ml. ethanol, 20 ml. ether, and 20 ml. ethanol for 30 minutes with each change of solvent. The combined extracts were reduced to a small volume by evaporation and the residue taken up in 20 ml. of hot saline to yield a clear solution, which was cooled and diluted with saline to the desired volume for bioassay.

The uterine weight response in immature mice was used in the bioassay of estrogen (Evans, Varney and Koch). All dilutions of samples were made with saline. Each sample was assayed at 4 levels, 5 mice for each level. Each mouse received 0.5 ml. sample twice daily for 3 days. After subtraction of the average uterine weight of control mice (saline injections), the uterine weights obtained were compared with a weight-response curve constructed from dilutions of the identical hormone-containing medium added to the flasks at the beginning of each set of experiments. The activity of the media, slices and protein precipitates were therefore compared directly with the media before incubation. It was found that the administration of hormone to the mice in aqueous solution yielded 2 to 3 times the response given by the same amount of estrogen administered in sesame oil as medium. An amount of 0.03 microgram of α-estradiol in saline per mouse yielded an average uterine weight increase above control weight of 25.1 mg. Where possible, at least two levels were used in calculation of activity. The errors were well within those given by Evans et al.

The samples of normal human prostate were obtained in the course of surgery in which removal of the normal gland was secondary to other operative measures. They were found to be normal on examination of microscopic sections.

DISCUSSION

Experiment 1 in Table 1 shows that estrogen is lost from the medium on incubation with prostatic slices. When the fate of the estrone was investigated, Experiment 2, it was found that the estrogen lost from the medium could be extracted from the slices. This removal

of estrogen from the medium appears not to be a simple diffusion or distribution process. The fact that heat-inactivated slices, Experiments 2 and 3, adsorb nearly equal quantities of estrogen suggests a physical adsorption process. It is conceivable that the water-insoluble, fat-soluble hormones are adsorbed by tissue lipids. Since the heat-coagulated protein of the medium contained no hormone activity it would appear that the adsorption of estrogens is not due to the formation of a hormone-protein complex.

TABLE 1. DISTRIBUTION OF ESTROGENS BETWEEN SLICES AND MEDIUM IN *in vitro* EXPERIMENTS

Experiment no.	Tissue	Wet wt. tissue mg.	Time[1] of incubation min.	Qo_2[2]	Volume[3] medium ml.	Estrogen added	Total estrogen added micrograms	Estrogen in medium after incubation[4] micrograms	Estrogen in slices micrograms	Estrogen in protein precipitates micrograms
1.	Human prostate	146.5	135	2.7	7.0	none	none	0.00	—	—
	Human prostate	241.0	135	2.8	10.5	estrone	1.05	0.46	—	—
	Human prostate	116.0	135	2.5	3.5	estrone	3.50	1.30	—	—
	Human prostate	134.5	135	2.7	7.0	α-estradiol	0.70	0.39	—	—
	Human prostate	57.1	135	2.4	3.5	α-estradiol	3.50	1.96	—	—
2.	Human prostate	315.0	120	1.5	10.5	none	none	0.00	0.00	0.00
	Human prostate	491.2	120	1.3	17.5	estrone	3.50	2.13	1.52	0.00
	Human prostate	147.0	120	1.3	7.0	estrone	7.00	5.80	1.75	0.00
	Human prostrate previously heated to 100°	140.0	120	0.1	3.5	estrone	7.00	2.70	—	0.00
3.	Rat liver	278.1	120	6.0	10.5	none	none	0.00	0.00	0.00
	Rat liver	209.5	120	5.4	7.0	α-estradiol	7.00	0.00	0.00	0.00
	Rat liver	91.0	55	5.8	7.0	α-estradiol	7.00	2.10	0.00	0.00
	Rat liver previously heated to 100°	221.0	120	0.0	7.0	α-estradiol	7.00	1.54	4.75	0.00

[1] Time does not include 10 minute temperature equilibration period.

[2] $Qo_2 = \frac{\text{microliters } O_2 \text{ taken up}}{\text{mg. dry wt.} \times \text{time (hrs.)}}$.

[3] Single vessels contained 3.50 ml. medium. All volumes reported are multiples of this figure since it was necessary to pool contents of two or more identically arranged vessels to yield enough estrogen for bioassay.

[4] Estrogenic activity expressed in terms of form of estrogen added.

This adsorption effect has to our knowledge not been described. In view of the data presented in this paper it is obvious that assay of the heat-deproteinized media alone will not necessarily yield a valid measurement of estrogen destruction. We therefore recommend that the entire contents of the Warburg vessel be extracted and assayed in order to determine the net gain or loss of estrogenic activity. These findings do not invalidate the *in vitro* studies of Heller and Singher et al. in which the combined medium and homogenized tissue was injected.

A second conclusion afforded by the data in the table concerns the ability of prostate slices to create or destroy estrogenic activity. There was no *net* gain or loss of estrogenic activity within the limits of error

of the bioassay. In this regard it is of interest to note that Heller found that uterus, spleen, kidney, ovary and heart increased the activity of estrone. The presence of hormone had no significant effect on the rate of respiration of the prostate slices.

These data are of some interest in connection with the *in vitro* action of androgen and estrogen on the growth and metabolic characteristics of the prostate gland previously demonstrated by many, including Moore and Price, Huggins and Clark, and Barron and Huggins, and with the therapeutic effect of estrogen on prostatic cancer demonstrated by Huggins and Hodges.

We believe that the method of bringing estrone and α-estradiol into aqueous solution described here is superior to the use of buffer-diluted acetone or alcohol solutions of hormones. With the solution prepared as described we have noticed much higher rates of destruction of α-estradiol by normal rat liver slices than those reported by the Memorial Hospital group, who found that 100 mg. (wet weight) of rat liver slices would just inactivate completely 3 micrograms of α-estradiol in 2 hours at 38° C. The data of experiment 3 in the table show that 91 mg. of slices inactivated 5.0 micrograms of α-estradiol in less than one hour, indicating a rate of destruction three times greater than that found by Singher et al.

SUMMARY

Human prostate slices remove large amounts of estrone or α-estradiol from a buffered suspending medium containing these hormones on incubation at 38° C. Estrone removed from the medium is apparently adsorbed, since it can be recovered by extraction of the slices with ethanol and ether. The same effect is given by heat-inactivated prostate or rat liver slices, indicating that the adsorption is not a metabolic process. The magnitude of this effect is large and would affect the validity of conclusions regarding estrogen formation or destruction based on bioassay of the suspending medium alone after incubation of tissues with hormone-containing medium. Slices of normal human prostate do not cause a *net* decrease or increase of the estrogenic activity of added estrone or α-estradiol, nor do these hormones have any effect on the rate of respiration of the slices.

REFERENCES

Barron, E. S. G., and C. Huggins: *J. Urol.* 51: 630. 1944.
Evans, J. S., R. F. Varney and F. C. Koch: *Endocrinology* 28: 747. 1941.
Heller, C. G.: *Endocrinology* 26: 619. 1940.
Huggins, C., and P. J. Clark: *J. Exper. Med.* 72: 747. 1940.
Huggins, C., and C. V. Hodges: *Cancer Research* 1: 293. 1941.
Moore, C. R., and D. Price: *Proc. Soc. Exper. Biol. & Med.* 28: 38. 1930.
Singher, H. O., C. J. Kensler, H. C. Taylor, Jr., C. P. Rhoads and Klaus Unna: *J. Biol Chem.* 154: 79. 1944.
Twombly, G. H., and H. C. Taylor: *Cancer Research* 2: 811. 1942.
Zondek, B., and J. Sklow: *Proc. Soc. Exper. Biol. & Med.* 46: 276. 1941.

CONCERNING THE MECHANISM OF DIESTRUM DURING LACTATION IN THE ALBINO RAT

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IN THE past ten years, much research work has been devoted to the problem of lactation anestrum. The lactogenic hormone of the pituitary, "prolactin," has been shown to have a definite inhibitory action on the estrous cycles of rat and mouse (Dresel; Lahr and Riddle; Nathanson and Fevold; Nathanson, Fevold and Jennison) and recent publications have emphasized the so-called luteotropic properties of prolactin; i.e., the maintenance of functional corpora lutea (Evans, Simpson, Lyons and Turpeinen).

In the light of these results, it would seem that these two properties of the lactogenic hormone provide a satisfactory explanation of the behavior of the gonads during lactation without the need of postulating changes in the gonadotropic activity of the hypophysis during this period. That the matter is not so simple is indicated by the following considerations.

Ten years ago our attention was directed to the structural changes of the pituitary during lactation in the rat. We were able to show that in this species castration at delivery does not result in the classical castration picture of the pituitary, provided suckling is allowed; whereas typical castration changes regularly make their appearance if the young are removed from the mother (Desclin, 1936). In the light of these results and of the well-known fact that the structural changes of the castration pituitary are correlated with a definite augmentation of its gonadotropic potency, it seemed justified to conclude that lactation has an influence on the gonadotropic mechanism of the pituitary itself. In other experiments we studied the structure of the ovaries of immature rats grafted into the kidneys of a series of spayed lactating rats in comparison with the contralateral ovary of the same infantile donors implanted into the kidney of a series of spayed rats whose young had been removed. In the first group the implants remain small and show exclusively young follicles and no corpora lutea, whereas in the second group the implants grew tremendously and were filled with corpora lutea. This result we interpreted as the physiological expression of changes in the hypophysis under the influence of suckling (Desclin and Gregoire, 1937).

This interpretation differs greatly from that which might be in-

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ferred from the above-mentioned publications. It postulates, besides a peripheral action of the lactogenic hormone on the gonads, a primary change in the gonadotropic function of the pituitary during lactation.

It is, however, possible that during lactation prolactin exerts its influence not only on the gonads but also on the pituitary basophils themselves. In this case, the pituitary changes would be merely a secondary phenomenon.

To attempt to solve this question we undertook the experiments which are reported here. In a first group of animals, we extended our earlier researches to the study of the influence of suckling on the structure of the pituitary in spayed rats whose main galactophoric ducts had been cut off to prevent escape of milk and thus to rule out the consequent metabolic drain. The other series are devoted to the possible influence of injected prolactin on the structure of the hypophysis in spayed rats.

MATERIAL AND METHODS

Female albino rats from our stock colony, eight months old at the onset of the experiment, were used.

In the first experiment ten pregnant rats were operated upon during the week before delivery. Small incisions were made laterally from the nipples and, through a funnel dissected underneath the skin, the main ducts of all mammary glands were sectioned. Every pregnant rat had as a wet nurse an unoperated mother to suckle the young of the experimental female. A given litter was placed alternatively one day with an operated mother, the next day with the wet nurse. To insure an intense and continuous stimulation of the nipples, as many young as possible, even up to 20, were employed. In the operated mother this energetic suction resulted in a striking elongation and reddening of all the nipples. The rats whose milk ducts had been severed were also spayed on the day of delivery. They were sacrificed 10 days later. The young which had been nursing on presumably dry nipples during the last twenty-four hours were also killed and their stomach carefully examined to check the completeness of the section of the ducts, and this was likewise controlled in the mother at post mortem examination. The mammary glands and hypophysis were preserved for histological study.

For the other experiment devoted to the action of prolactin on the structure of the pituitary 90 animals were used. They were divided into three groups:

The first, designed to test the activity of the extract administered, comprised 30 rats. Ten were injected daily with 2 mgr. prolactin. Ten others received a dosage of 3 mgr. daily. Ten uninjected rats served as controls. The injections lasted 10 days. On the fifth day the abdomen was opened and a silk thread introduced in both uterine horns, care being taken that the thread passed through the lumen. The animals were killed the day after the last injection.

In the second group 10 castrated animals received 3 mg. per day for 10 days. The injections were started on the day of operation. On the fifth day the uterine horns were traumatized as in group 1. Ten rats uninjected but

treated in exactly the same way served as controls. All animals were killed on the eleventh day after the onset of the experiment.

The third group consisted of 20 pregnant rats to simulate as nearly as possible the conditions in spayed lactating animals. The rats were spayed the day of delivery and injected daily for ten days with 3 mgr. of prolactin, beginning on the day of operation. They were sacrificed the day after the last injection. Twenty uninjected pregnant rats similarly spayed at delivery and killed eleven days later served as controls. In both control and experimental animals the young were removed from the mother immediately after delivery.

The prolactin used was a commercial preparation made from sheep pituitaries.[1] Tested by the crop gland method it was reported to have a potency of 12 Riddle units per mgm. Supplied as a dry powder, the material was dissolved in distilled water, adjusted with 1/10 N NaOH to pH 10 for better solubility. A solution containing 10 mgr. per cc. was uniformly employed. During the period of the experiment the solution, which was never used longer than three days, was kept in the refrigerator.

At the end of the experiment the uterus was examined microscopically for detection of deciduomata. The entire genital tract, including ovaries and vagina, were removed and fixed in Bouin-Holland-Sublimate for microscopical examination as were also thyroid, adrenals, mammary glands and hypophysis. The hypophysis was stained by the Mallory-Azan method, the other organs by the trichrome method of Masson. The adrenals were weighed after fixation at their passage in the 90% alcohol.

We have evaluated the activity of the thyroid by measuring the cellular height in a hundred vesicles of representative sections through the middle of the gland.

RESULTS

I. *Influence of suckling in female albino rats after resection of the main galactophoric ducts.*

The experimental animals, spayed at delivery,[2] generally took care of their young, despite the frequent exchanges from mother to mother. When a rat killed her litter, as occasionally happened, she was immediately discarded. In three of the ten operated animals a number of the nipples had become necrotic by the end of the experiment, probably through interference with the blood supply, and had not been used by the young. These cases were likewise discarded.

The seven remaining animals formed the basis of our conclusions, as they showed on careful post-mortem examination a complete transsection of the galactophoric ducts and the stomachs of the young were empty.

Selye and coworkers (1934) have shown that after resection of the

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[1] This preparation was generously supplied by the Glaxo Laboratories, Ltd., Greenford, Middlesex, England, through the courtesy of Dr. F. A. Robinson to whom our best thanks are due.

[2] These experiments have been the subject of a preliminary report by Desclin &. Gregoire. *Compt. rend. Soc. de Biol* in press.

milk ducts the secretion continues (provided suckling is allowed), despite the fact that the milk cannot escape from the glands. The study of the mammary glands in our material confirms their findings. At corresponding periods of time the contrast is striking between the mammary glands of the animals whose nipples have been suckled as compared with those of the weaned controls. In the former the alveoli are still distended with milk without any sign of regression, whereas in the latter the alveoli have collapsed and reveal a shrunken appearance and definite signs of regression (fig. 1 and 2). Our animals have been spayed at delivery and killed ten days later. Under these conditions mothers which have not been suckled showed very definite castration signs in their hypophysis. The basophils had increased in number and dimensions with very conspicuous nucleoli (fig. 3). The picture of the pituitary in the animals which were suckled was entirely different. In these no castration cells made their appearance and there was no significant change in the features of the basophils (fig. 4.) The eosinophils looked different and the proportion of degranulated forms was much higher than in the controls after weaning. Many cells in Dominici preparations in which the toluidin blue reveals true basophilia, showed basophilic inclusions which were ribonucleic in nature, as we have shown previously. These inclusions are lacking in animals which have not been suckled.

II. *Influence of prolactin on the pituitary in spayed normal and post partum animals.*

A. *Preliminary experiment in non-castrated animals.*

Tables I and II (normal animals) give a summary of the results for the injected animals and the uninjected controls. It may be seen that with a treatment of 2 mgr. daily the uterine horns were hyperemic, their volume was augmented and, in 5 animals out of 10, voluminous deciduomata formed. With a dose of 3 mgr. daily the appearance of the uterus was quite the same but 9 animals in 10 reacted with deciduomata formation. The controls showed uterine horns which were definitely thinner with no reaction at the level of the threads. Some of the uteri were of translucid appearance, and were filled with liquid as in oestrus, phenomena entirely lacking in the injected animals. The vaginal smear never showed signs of oestrus in the injected animals and the histologic study of the vagina revealed variable degrees of mucification which in some cases was as intensive as in pregnancy (fig. 5). Some of the uterine horns which did not react with deciduomata formation showed nevertheless a folded epithelium characteristic of the luteal phase. The ovaries of all the injected animals contained large corpora lutea, definitely larger than in the controls and composed of larger individual cells. The interstitial tissue was very scant in most cases and in others it was more developed but

never more abundant than in the controls. The ovaries had the same appearance as in pregnancy.

As a rule the follicles appear normal. In a few ovaries cysts may be found, bordered by an irregular line of luteal cells which often accumulate at one side to give crescent-shaped formations. These formations seem to have a thecal origin though we did not succeed in finding rests of the granulosa in the cavity of these cysts (fig. 6). The ovarian picture seems to indicate that the extract used did not contain much follicle stimulating hormone and was probably contaminated with small quantities of the luteinizing hormone.

The adrenals were not significantly hypertrophied in the injected animals, as seen from the data given in table II.

The activity of the thyroid as judged by the cellular height remained likewise the same in injected as in non-injected animals. Our extract seemed thus to contain very little thyrotropic and corticotropic hormone.

TABLE I. PART 1. INFLUENCE OF THE INJECTION OF PROLACTIN ON UTERUS AND MAMMARY GLAND IN NORMAL ANIMALS

Animals	Body-weight in gm.	Dose in mg.	Injections* started	Thread* through uterus	Sacrifice*	Deciduoma	Proliferation of the mammary gland	Secretion process
1	235	2	14.3	19.3	25.3	+++	intense	absence
2	243	2	14.3	19.3	25.3	0	intense	marked
3	253	2	14.3	19.3	25.3	0	intense	absence
4	203	2	14.3	19.3	25.3	0	poor	slight
5	206	2	14.3	19.3	25.3	+++	intense	very slight
16	207	2	22.3	26.3	1.4	+++	intense	absence
17	202	2	22.3	26.3	1.4	0	intense	marked
18	198	2	22.3	26.3	1.4	+++	marked	absence
19	188	2	22.3	26.3	1.4	0	poor	slight
20	214	2	22.3	26.3	1.4	+++	intense	absence
21	200	3	25.3	29.3	4.4	+++	very intense	slight
22	218	3	25.3	29.3	4.4	+++	very intense	slight
23	227	3	25.3	29.3	4.4	+++	intense	absence
24	189	3	25.3	29.3	4.4	+++	very intense	very slight
25	197	3	25.3	29.3	4.4	+++	intense	absence
26	203	3	28.3	1.4	7.4	0	intense	absence
27	245	3	28.3	1.4	7.4	+++	very intense	absence
28	182	3	28.3	1.4	7.4	+++	well marked	very slight
29	184	3	28.3	1.4	7.4	+++	intense	absence
30	210	3	28.3	1.4	7.4	+++	very intense	absence
Non-injected controls								
6	223	—	—	21.3	26.3	0	poor	absence
7	178	—	—	21.3	26.3	0	very poor	absence
8	208	—	—	21.3	26.3	0	poor	absence
9	180	—	—	21.3	26.3	0	very poor	absence
10	168	—	—	21.3	26.3	0	poor	absence
11	202	—	—	22.3	27.3	0	poor	absence
12	205	—	—	22.3	27.3	0	poor	absence
13	224	—	—	22.3	27.3	0	poor	absence
14	225	—	—	22.3	27.3	0	poor	absence
15	204	—	—	22.3	27.3	0	poor	absence

* First number indicates day-second number the month.

TABLE I, PART 2. INFLUENCE OF THE INJECTION OF PROLACTIN ON UTERUS AND MAMMARY GLANDS IN CASTRATED ANIMALS

Animals	Castration	Body weight in gr.	Dosage in mgr.	Injections started	Thread through uterus	Sacrificed	Deciduomata	Proliferation of mammary gl.	Secretion process
31	29.3	196	3	29.3	2.4	8.4	0	marked	very intense
32	29.3	214	3	29.3	2.4	8.4	0	marked	intense
33	29.3	245	3	29.3	2.4	8.4	0	marked	intense
34	29.3	200	3	29.3	2.4	8.4	0	marked	intense
35	29.3	182	3	29.3	2.4	8.4	0	marked	very intense
41	1.4	219	3	1.4	5.4	11.4	0	marked	intense
42	1.4	172	3	1.4	5.4	11.4	0	marked	intense
43	1.4	167	3	1.4	5.4	11.4	0	marked	intense
44	1.4	191	3	1.4	5.4	11.4	0	marked	very intense
45	1.4	178	3	1.4	5.4	11.4	0	marked	intense
Castrated controls									
36	30.3	170	—	—	3.4	9.4	0	atrophic	absent
37	30.3	171	—	—	3.4	9.4	0	atrophic	absent
38	30.3	172	—	—	3.4	9.4	0	atrophic	absent
39	30.3	196	—	—	3.4	9.4	0	atrophic	absent
40	30.3	179	—	—	3.4	9.4	0	atrophic	absent
46	2.4	195	—	—	6.4	12.4	0	atrophic	absent
47	2.4	224	—	—	6.4	12.4	0	atrophic	absent
48	2.4	170	—	—	6.4	12.4	0	atrophic	absent
49	2.4	150	—	—	6.4	12.4	0	atrophic	absent
50	2.4	160	—	—	6.4	12.4	0	atrophic	absent

TABLE II. ADRENAL WEIGHTS AND THYROID ACTIVITY OF PROLACTIN TREATED ANIMALS

Weight of the adrenals in mg. calculated for 200 gm. body weight				Mean height of the epithelium in 100 vesicles of thyroid in μ			
Normal Controls	Normal Injected	Spayed Controls	Spayed Injected	Spayed Injected	Spayed Controls	Normal Injected	Normal Controls
35	39	50	48	8, 77	11, 72	11, 10	11, 78
45	45	35	34	9, 11	9, 07	12, 52	12, 03
37	41	38	35	8, 19	9, 65	12, 72	11
39	46	38	48	8, 29	11, 02	12, 56	10, 68
42	34	45	43	9, 30	9, 08	12, 38	11, 84
45	48	48	36	8, 80	12, 40	9, 47	13, 09
47	41	42	49	9, 01	10, 85	10, 48	10, 04
56	46	46	40	9, 50	9, 91	10	11, 06
31	36	46	36	10, 85	10, 64	10, 28	11, 27
40	41	40	43	9, 68	8, 70	9, 22	10, 49
	48						
	34						
	35						
	48						
	43						
	36						
	49						
	39						
	36						
	43						
Mean 41, 7	41, 4	42, 8	41, 2	9, 15	10, 30	11, 07	11, 32
probable error of the mean ±1, 49	±0, 77	±1, 06	±1, 25	±0, 16	±0, 26	±0, 29	±0, 39

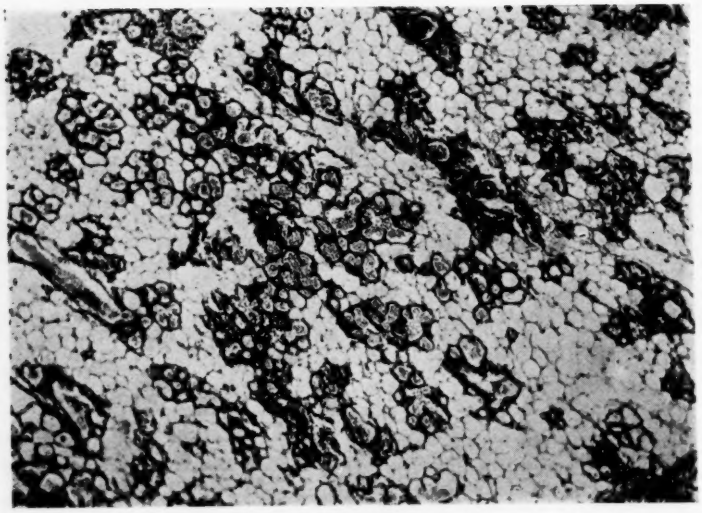

FIG. 1. Mammary gland of a female spayed at delivery whose ducts have been cut. Killed ten days later. Alveoli distended with milk. Suckling has been allowed.

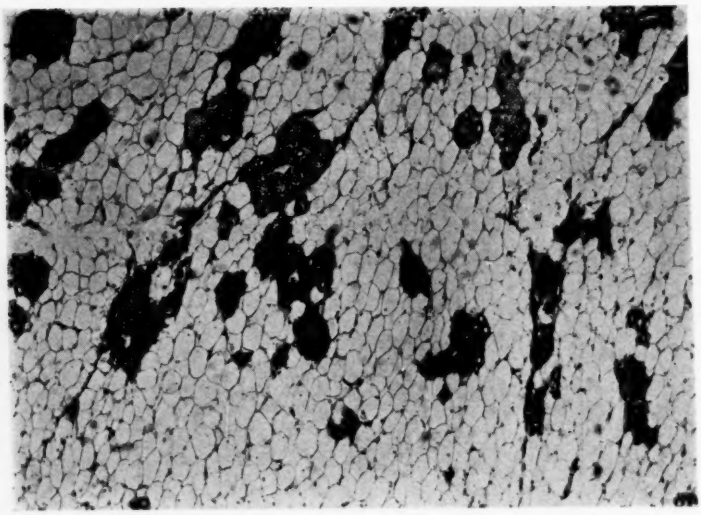

FIG. 2. Mammary gland of a female spayed at delivery whose young have been removed. The gland is regressing. Compare with fig. 1. Same magnification. This animal has been killed 10 days after delivery.

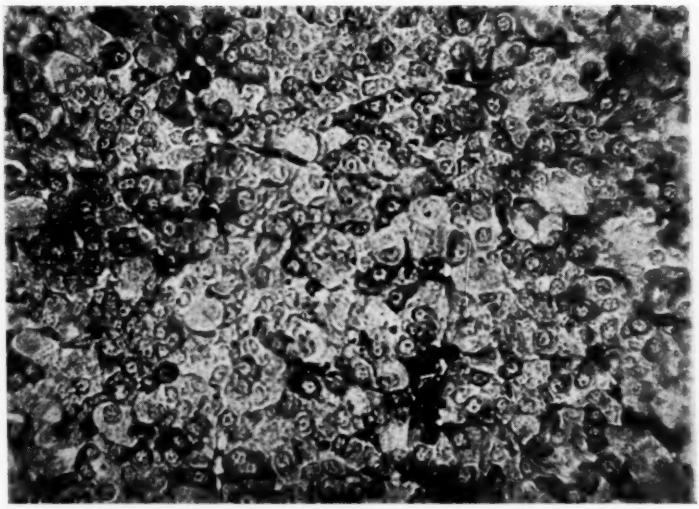

FIG. 3. Pituitary of a female spayed at delivery whose young have been removed. Killed ten days later. Castration picture. Numerous basophils which appear as big clear cells on the photograph.

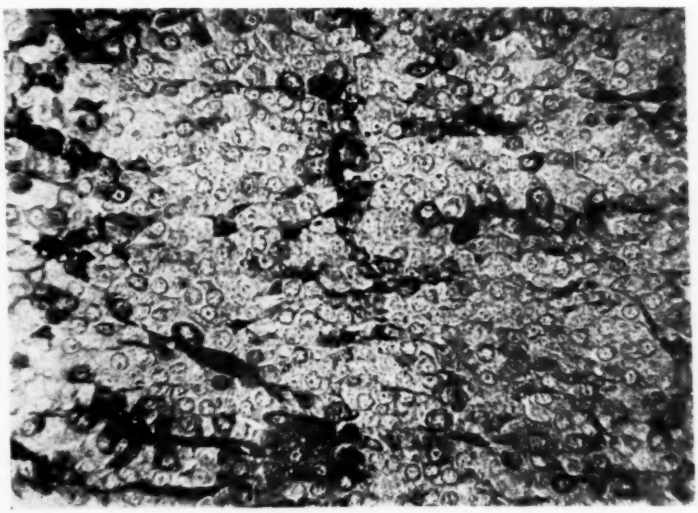

FIG. 4. Pituitary of a female spayed at delivery. The galactophoric ducts have been cut. Suckling has been allowed. Killed after 10 days. The castration changes did not appear in the pituitary. Note the degranulation of eosinophils. Compare with fig. 3. Same magnification.

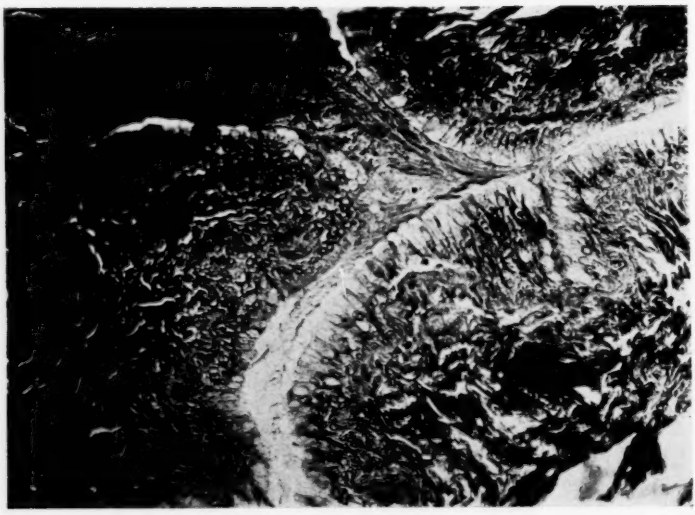

FIG. 5. Normal female rat injected daily with 3 mg. Prolactin. Killed after 10 days. Vaginal mucification comparable to the pregnancy picture.

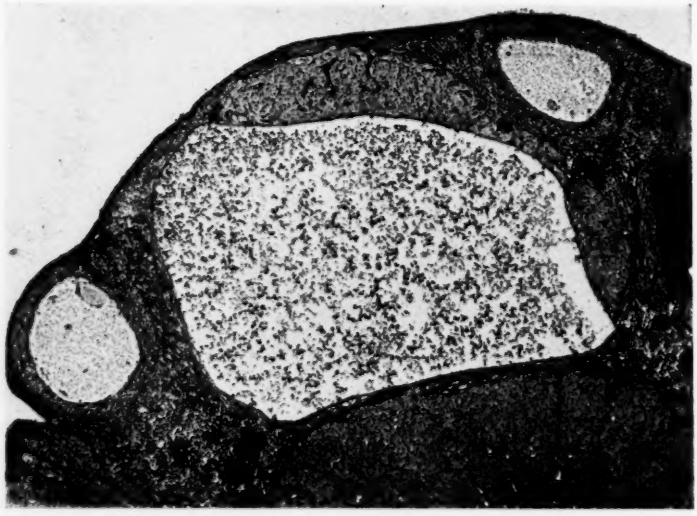

FIG. 6. Normal female injected daily with 3 mg. Prolactin. Killed after 10 days. As a rule, the ovarian follicles are not affected. Sometimes, follicular cysts are found with a partial luteinization of the theca interna.

The study of the mammary glands showed very different results in the injected animals as compared with the controls. Whereas the latter exhibited only a few ducts with very few lobules, the injected animals show an intense lobular proliferation. As a rule, the alveoli were not releasing secretion products into the lumina, which remained

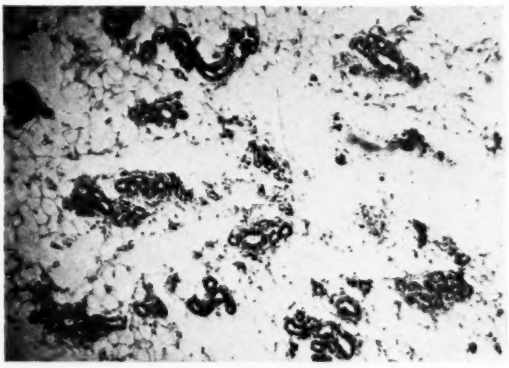

FIG. 7. Mammary gland of a normal female control.

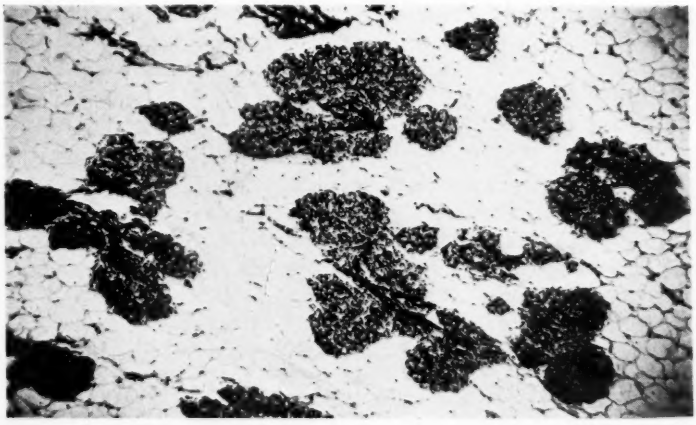

FIG. 8. Mammary gland of a normal female injected daily with 3 mg. Prolactin. Intense lobular proliferation. No secretion products in the alveoli. Compare with fig. 7. Same magnification.

empty. These products remained in the cells where they accumulated as droplets or coalesced into large fat drops at the top of the cell. In a few cases some liquid distended the alveoli and were in exceptional cases found in the ducts. Usually the mammary gland had a development and appearance comparable to what may be found during the last third of pregnancy (fig. 7 and 8).

These results show clearly that the prolactin used had a definite action on the mammary glands. It had also a definite luteotropic action for which a daily dosage of 3 mgr. was found to be optimal. In the rest of our experiments this daily dose of 3 mgr. was accordingly used.

B. *Spayed normal animals.*

As may be seen from Table I, the genital tract was found regularly atrophic as well in the injected animals as in the controls and no deciduomatous reaction could be detected. There is also no significant difference between the adrenals of injected and uninjected animals (Table II).

The study of the cellular height in the thyroid gave interesting results. Castration brought about a very definite state of rest in the thyroid. The cells had a mean height of 10.30μ as compared with 11.32μ for the normal. The statistical analysis showed that this difference is significant. This result was not unexpected. More surprising was the difference between injected and uninjected animals. The prolactin injections seemed to have depressed the thyroid activity of the spayed animals whose cellular height became 9.15μ as compared with 10.30μ in the uninjected controls. The same depressive tendency could already be suspected in the normal animal but the difference between injected and non-treated animals was not significant. In the spayed animals, the difference became definitely significant.

This result, besides showing that the extract used did not contain thyrotropic hormone, is difficult to understand. It is worth mentioning in this connection that Dr. Gregoire, in this laboratory, studying the action of lactation on thymus atrophy with the same material, found that the thyroid of the lactating animal was definitely more active. But he also found that in animals whose ducts had been sectioned the thyroid was at rest, in spite of the intensive stimulation of the nipples. I am at present at a loss to find an adequate explanation of this fact and of my own observations of a depressive action of prolactin on the thyroid.

The mammary glands of the spayed controls were after ten days definitely regressing and consisted only in a few naked ducts scattered in fat tissue (fig. 9). In the injected animals the picture was entirely different. The mammary gland was still very well developed and showed numerous acini. The alveoli were actively secreting and distended with milk. The ducts also were filled with secretion products (fig. 10).

There was a striking contrast between this finding and the picture we have found in the normal animal injected with the same dosage for the same period of time (fig. 8). In the latter the secretion products were merely accumulating within the cells and were not released into the lumen, whereas in the spayed animals no secretion products accumulated within the cells but were immediately released and the alveoli and ducts filled with liquid.

FIG. 9. Spayed control. Killed 10 days after spaying. Mammary gland atrophic. Compare with fig. 7. Same magnification.

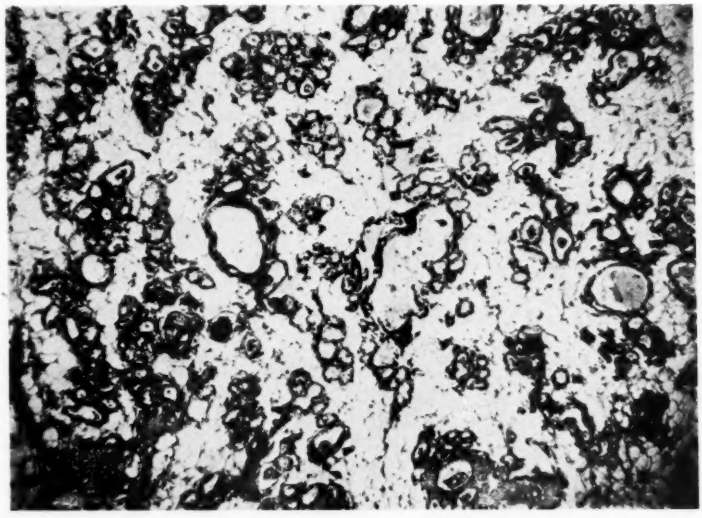

FIG. 10. Mammary gland of a spayed female rat injected daily with 3 mg. Prolactin. Killed 10 days after spaying. Intensive lobular proliferation. The alveoli and ducts are distended with secretion products. Compare with fig. 9 and 8. Same magnification.

It seems that a certain inhibitory action on the secretory activity of the mammary gland must be exerted by the ovary under the action of prolactin. This result is in consonance with observations of Lyons (1941), Laqueur (1943) and Zeckwer (1944).

The main object of this experiment was to see if the administration of an active lactogenic preparation should have an influence on the structure of the pituitary.

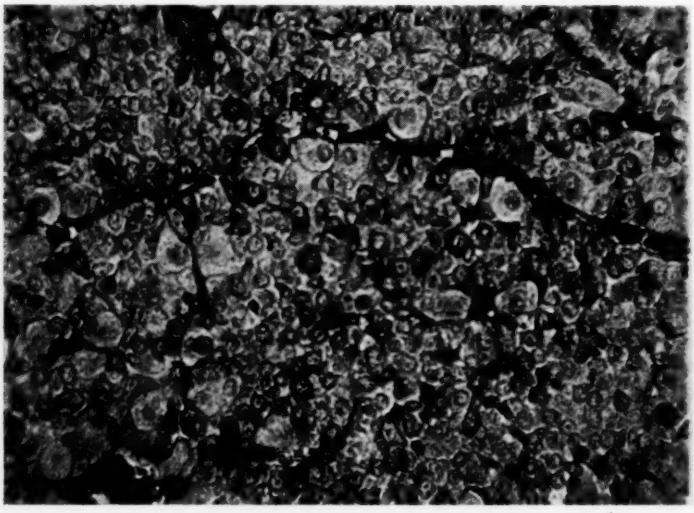

FIG. 11. Pituitary of a spayed female rat injected daily with 3 mg. of prolactin. Killed 10 days after spaying. The treatment did not affect the castration picture of the pituitary. Compare with the structure of a spayed suckling female fig. 3.

The controls regularly showed the well-known structure of the castration pituitary. After ten days the vacuolated basophils had not yet made their appearance but there was a definite increase in size and number of basophils with prominent maculae. The eosinophils did not show alterations from the normal and the majority of them were well filled with granules.

The study of the injected animals shows pituitaries entirely comparable to those of the controls. The basophils were as numerous and showed exactly the same appearance (fig. 11).

C. *Pregnant animals spayed at delivery.*

To realize exactly the same conditions as those of spayed lactating animals, we also injected with prolactin animals spayed at delivery and treated from the day of operation.

As the results were exactly the same as those of the previous group;

i.e., the injections had not the least influence on the structure of the pituitary, it is unnecessary to go into detail concerning them.

DISCUSSION

Histologic changes in the pituitary during lactation were mentioned in the rat as early as 1932 by Haterius, who attributed them to the influence of the persisting corpora lutea of lactation. This concept cannot be maintained, however, in view of our later results which demonstrated that the lactation changes of the pituitary persist after castration in the rat provided suckling is allowed (Desclin, 1936).

Neither may they be considered as unspecific changes connected with the particular metabolic conditions during lactation and resulting from the new demands imposed on the organism for the elaboration of considerable quantities of milk. Reduction in size of the litter does not affect appreciably the pituitary picture (Desclin, 1945). The experiments reported here show that the mere stimulation of the nipples without milk loss is able to maintain the pituitary changes characteristic of lactation. These results are in accordance with previous findings of Turner and Reece who showed that the mere act of suckling without milk removal influences the prolactin content of the rat pituitary.

The structural changes associated with lactation seem to be the proper morphologic expression of the hypophyseal activity. During lactation a high proportion of the eosinophils are degranulated and show signs of active secretion-enlargement of the Golgi area, basophilic ribonucleic inclusions in the cytoplasm (Desclin, 1940, 1945; Everett, 1945). It may be considered as an argument in favor of the elaboration of prolactin through the eosinophils. Further support for this idea may be found in the work of Schooley and Riddle, A. Asimov, and Friedman and Hall.

Another characteristic feature of the pituitary during lactation is the change in the basophils. These cells do not hypertrophy either in size or in number after castration as they regularly do in the non-suckled animal (Desclin 1936, 1945). This is also the case in spayed animals whose milk ducts have been cut, as shown in the present paper.

This result seems to indicate that lactation interferes with the gonadotropic activity of the pituitary. This was our explanation for the results of experiments in which we implanted infantile ovaries into the kidneys of spayed lactating and non-lactating rats. In lactating animals the implants were small and contained young follicles without corpora lutea. In the rats whose young had been removed, the implants had enormously enlarged and contained numerous corpora lutea (Desclin and Gregoire, 1937). It seemed to us that these facts have a certain significance for the problem of lactation anoestrum.

In connection with this problem, the role of prolactin has of late been particularly stressed. It has been shown by many authors that the injection of the lactogenic hormone inhibits the oestrous cycles in rat and mouse (Dresel, Lahr, and Riddle, Nathanson and Fevold, Nathanson, Fevold and Jennison). The study of the action of prolactin in birds, where its injection brings about a definite atrophic condition of the gonads (Lahr and Riddle), led the authors to the conclusion that prolactin is an antagonist of F.S.H. and to see in this property an explanation of the anti-estral action of prolactin in the mammal.

Though the presence of corpora lutea in the ovaries of prolactin-treated animals had already been mentioned in these publications, it remained for more recent researches to insist on the luteotropic action of the lactogenic hormone (Evans, Simpson, Lyons and Turpeinen and others). The extract used in our experiments has also shown this action on the corpora lutea.

These properties of prolactin could, of course, explain the results of our ovarian grafts through a peripheral action of the prolactin secreted by the animal's own pituitary under the influence of suckling. However, if prolactin, acting at the periphery, were the only factor involved in lactation dioestrum, the absence of castration cells in the pituitary of spayed lactating rats should be a consequence of its interaction with the F.S.H. and prolactin injections should prevent their appearance in the pituitary of rats spayed at delivery whose young have been removed. The results of the experiments reported here show conclusively that this is not the case.

Under these conditions, there must be a direct influence of suckling on the pituitary basophils themselves and consequently on the gonadotropic activity of the hypophysis. Preliminary results of experiments which are not yet completed seem to indicate that the gonadotropic content of the pituitary is definitely influenced by lactation. These facts have to be considered in the explanation of lactation dioestrum.

SUMMARY

In spayed postpartum rats whose main milk ducts had been cut, the mere stimulation of the nipples by suckling prevented the appearance of castration cells in the pituitary. The injection of prolactin in spayed normal or in spayed postpartum animals whose young had been removed did not prevent the appearance of castration cells in the anterior pituitary. The bearing of these facts on the problem of lactation dioestrum was discussed.

REFERENCES

Azimov, G. I. and A. D. Altman: *Compt. rend. Acad. d. sc.* U.R.S.S. 20: 621. 1938.
Desclin, L.: *Compt. rend. Soc. de biol.* 122: 447. 1936.
Desclin, L.: *Compt. rend. Soc. de biol.* 133. 457. 1940.
Desclin, L.: *Arch. de biol.*, Paris 56: 261. 1945.
Desclin, L., and Ch. Grégoire: *Compt. rend. Soc. de biol.* in press 1945.

DRESEL, I.: *Science* 82: 173. 1935.
EVANS, H. M., M. E. SIMPSON, N. R. LYONS AND K. TURPEINEN: *Endocrinology* 28: 933. 1941.
EVERETT, N. B., AND B. L. BAKER: *Endocrinology* 37: 83. 1945.
FRIEDMAN, M. H., AND S. R. HALL: *Endocrinology* 29: 179. 1941.
GRÉGOIRE, CH.: *J. Endocrinol.* in press 1946.
HATERIUS, H. O.: *Anat. Rec.* 54: 343. 1932.
LAHR, E. L., and O. RIDDLE: *Proc. Soc. Exper. Biol. & Med.* 34: 880. 1936.
LAHR, E. L., R. W. BATES, AND O. RIDDLE: *Am. J. Physiol.* III: 361. 1935.
LAQUER, G. L.: *Endocrinology* 32: 81. 1943.
LYONS, W. R.: *Endocrinology* 28: 161. 1941.
NATHANSON, I. T., AND H. L. FEVOLD: *Endocrinology* 22: 86. 1938.
NATHANSON, I. T., AND D. B. JENNISON: *Proc. Soc. Exper. Biol. & Med.* 36: 481. 1937.
REECE, R. P., AND C. W. TURNER: *Proc. Soc. Exper. Biol. & Med.* 35: 621. 1937.
SELYE, H., AND T. MCKEOWN: *Surg., Gynec. & Obst.* 59: 886. 1934.
SELYE, H., AND T. MCKEOWN: *Anat. Rec.* 60: 323. 1934.
ZECKWER, I. T.: *Arch. Path.* 38: 99. 1944.

MOBILIZATION OF ALKALINE PHOSPHATASE IN THE UTERUS OF THE MOUSE BY ESTROGEN[1,2]

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A MARKED increase in the amount of alkaline phosphatase present in the uterus of the mouse following administration of estrogen has been reported in a previous paper (Atkinson and Elftman, 1946). The injection of estrogen into the ovariectomized mouse is followed by a pronounced increment of phosphatase in the uterine glands and lumenal epithelium and in the circular layer of the myometrium. Neither androgen nor progesterone produce this effect.

The purpose of the experiments here described is to study the relation between the amount of estrogen administered and the degree of mobilization of alkaline phosphatase. These experiments also provide data concerning the time of appearance and the duration of the response. With this information at hand it is possible to bring the alkaline phosphatase mobilizing effect of estrogen into at least temporal relation to its other physiological actions.

METHODS

In order to minimize individual variation, mice of the Swiss albino strain only were used in these experiments. Fifty young adults were ovariectomized. Following operation a minimum of one week was allowed for uterine regression before further treatment was instituted. At the end of this period the animals were divided into several groups for subsequent study.

Estradiol dipropionate was dissolved in sesame oil in such concentration that the daily dose was contained in 0.05 ml. of the oil. The minimal effective dose was determined by injecting twelve animals subcutaneously for three consecutive days with total doses of estrogen ranging from 0.15 to 3.0 micrograms (Table I). These animals were killed twenty-four hours following the final injection. In order to establish the temporal relations of the appearance and duration of the response, thirty mice were given single injections of 0.5 microgram of estrogen and were killed after intervals ranging from six hours to seven days (Table II). A final group of three mice was given 1.0 microgram of estrogen every other day for ten days, with autopsy twenty-four hours after the final injection.

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[2] The estradiol dipropionate used in this work was supplied through the courtesy of CIBA Pharmaceutical Products, Inc., Summit, New Jersey.

The animals were killed with illuminating gas and were autopsied immediately. The uterus was fixed in cold (5°–10°C.) 80% ethyl alcohol for 18 to 24 hours. After dehydrating and embedding in paraffin, sections were cut 5–7 micra in thickness. The tissue sections were incubated for three hours in a 1% sodium glycerophosphate substrate according to the technique of Gomori (1941) with the addition of 0.01 M magnesium sulphate. The sites of alkaline phosphatase activity are easily identified by the dark deposits of cobalt sulfide. The sections were not counterstained, in order to avoid obscuring the distribution of the deposit. They were mounted in clarite in the usual manner.

OBSERVATIONS

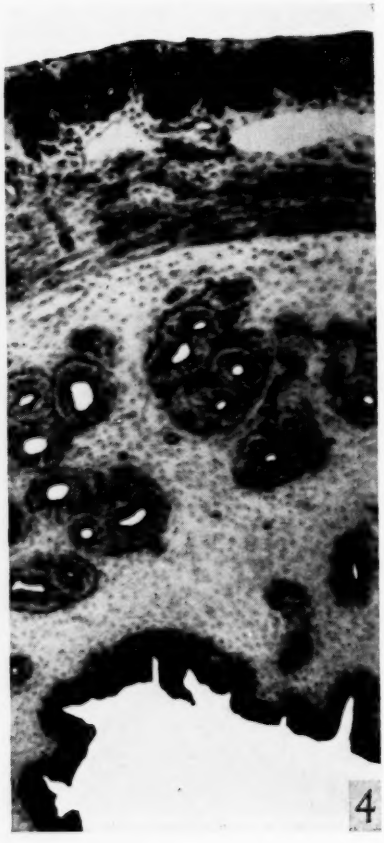
Uterus of the castrate

The distribution of alkaline phosphatase in the uterus of an untreated mouse which had been ovariectomized a week earlier is shown in figures 1 and 2. Although there is evidence of some phosphatase in the nuclei, its presence in the cytoplasm is essentially restricted to the longitudinal musculature and to the periglandular stroma. In contrast to the longitudinal musculature, the inner circular muscle layer contains but minimal amounts of phosphatase. There is also no evidence of any appreciable content of phosphatase in either the lumenal epithelium or in the glands. The phosphatase of the stroma in the mouse, when present, is localized in the region immediately surrounding the glands.

Minimal effective dose

The effect of various dosages of estradiol dipropionate on the alkaline phosphatase of the mouse uterus is summarized in Table I. The tissues which show a marked increase in phosphatase are the uterine epithelium, the glandular epithelium and the circular muscle (Figures 3 and 4). The epithelium of the uterus exhibits the most striking change. Even with a dosage of 0.15 microgram of estradiol dipropionate, distributed in three equal daily injections, two out of three mice exhibited distinct evidence of the presence of phosphatase in the apical portion of the epithelial cells. With larger doses, as may be seen from Table I, the density of the deposit increases, indicating a higher concentration of the enzyme. The increase in amount of estrogen administered leads not only to larger amounts of enzyme in the apical portion of the uterine epithelial cells but also to its appearance in the basal portion. A gradient of concentration rising towards the apex is, however, generally maintained. The enzyme content of the nucleus appears to rise with the increment in cytoplasmic alkaline phosphatase.

The epithelium of the uterine glands parallels the lumenal epithelium in the appearance of alkaline phosphatase following administration of estrogen. In addition to an apical-basal gradient in each cell, there is a decrease in the amount of phosphatase present as the gland is followed from its orifice to its blind termination.



FIGS. 1 and 2. Photomicrographs (×20 and ×140) of the uterus of an untreated ovariectomized mouse showing the distribution of alkaline phosphatase. The enzyme is present in considerable quantity only in the longitudinal muscle.

FIGS. 3 and 4. Photomicrographs (×20 and ×140) of the uterus of an ovariectomized mouse four days after the injection of 0.5 microgram of estradiol dipropionate. There is a marked increase in the alkaline phosphatase content of the lumenal epithelium, glands and circular muscle.

The absence, or minimal content, of alkaline phosphatase in the circular muscle layer of the castrate is in striking contrast to the high concentration of this substance in the adjacent longitudinal layer. Upon administration of estrogen, alkaline phosphatase appears in the cytoplasm of the circular muscle fibers. However, it does not rival in quantity the concentration which persists in the longitudinal muscle or that which is evoked in the lumenal epithelium. A somewhat larger dose of estrogen is needed to produce a definite reaction in the

TABLE I. RELATION BETWEEN THE QUANTITY OF ESTRADIOL DIPROPIONATE INJECTED AND THE AMOUNT OF CYTOPLASMIC ALKALINE PHOSPHATASE IN THE UTERUS OF THE OVARIECTOMIZED MOUSE

Total dose in micrograms. (Given in 3 equal daily doses)	No. of animals	Endometrium			Myometrium	
		Lumenal epith.	Glands	Stroma	Inner circ.	Outer long.
Untreated	5	0	0	0	0	+++
0.15	3	0−++	0−+	0	0	+++
0.3	3	++	+	0	+	+++
1.5	3	+++	++	0	++	+++
3.0	3	+++	++	0	++	+++

circular musculature than is required for significant phosphatase mobilization in the epithelium (Table I). The fibers of the longitudinal musculature contain large amounts of alkaline phosphatase in the castrate and show no discernible change after estrogen treatment.

Latent period and duration of response

The latent period and the persistence of the alkaline phosphatase response to estrogen were investigated by employing a single uniform injection of 0.5 microgram of estradiol dipropionate and observing the

TABLE II. EFFECT OF A SINGLE DOSE OF ESTRADIOL DIPROPIONATE (0.5 MICROGRAM) ON THE AMOUNT AND DISTRIBUTION OF CYTOPLASMIC ALKALINE PHOSPHATASE IN THE UTERUS OF THE OVARIECTOMIZED MOUSE

Time elapsed after injection	No. of animals	Endometrium			Myometrium	
		Lumenal epith.	Glands	Stroma	Inner circ.	Outer long.
Untreated	5	0	0	0	0	+++
6 hours	3	0	0	0	0	+++
12 hours	3	0	0	0	0	+++
24 hours	3	++	+	0	0−+	+++
2 days	3	+++	++	0	++	+++
3 days	3	+++	++	0	++	+++
4 days	6	+++	++	0	++	+++
5 days	3	++	+	0	++	+++
6 days	3	++	+	0	++	+++
7 days	3	0	0	0	0−+	+++

phosphatase content at increasing intervals after administration (Table II).

In the groups of animals sacrificed 6 hours and 12 hours after injection there was no demonstrable increase in alkaline phosphatase over that found in the castrate. Twenty-four hours after injection, however, the phosphatase in the uterine epithelium and in the glands was marked, with a less pronounced effect in the circular musculature. At 48 hours the phosphatase mobilization was maximal both in epithelium and in musculature. The latent period for the initial phosphatase response is consequently more than 12 hours and less than 24, with an increase in concentration during the following day. The latent period, as determined in this manner, includes not only the interval necessary for phosphatase mobilization but also the time occupied in the absorption of the estrogen.

Subsequent to the establishment of a maximal alkaline phosphatase content by the end of the second day following the single injection of estradiol dipropionate, no significant change was observed until the fifth day after treatment. The mice sacrificed at this time exhibit a diminution of alkaline phosphatase in the uterine and glandular epithelium but no significant decrease in the reaction of the circular musculature. Seven days after treatment the epithlium of the uterine lumen and glands has returned to the castrate condition, but the circular muscle gives evidence of a slight residuum of phosphatase.

Prolonged Treatment

Three mice were given 1.0 microgram of estradiol dipropionate every other day for ten days and were autopsied one day after the last injection. The distribution of alkaline phosphatase is similar. in general, to that described as the maximal effect in the previous series of experiments, but the intensity of the reaction is somewhat greater. This is especially noticeable in the augmentation of alkaline phosphatase in the basal portions of the epithelial cells. The amount of phosphatase in the periglandular stroma is noteworthy, resembling that found five or more days after the single injection.

DISCUSSION

Two problems are raised by the present observations on uterine alkaline phosphatase: (1) the nature of the mechanism by which estrogen effects the mobilization of phosphatase; and (2) the function of the enzyme after its appearance in the uterine tissues.

Our data have established several important relationships between estrogen and phosphatase which bear on the first problem. The studies on the minimal effective dose of estrogen indicate that there is a direct ratio between the level of hormone stimulation and the degree of enzyme response, since increasing amounts of phosphatase are found as the dose of estrogen is increased. The specific time-intervals in the enzyme response to a single dose of estrogen are probably true only for the particular dosage and the particular estrogen here

used. It is interesting to note, however, that the enzyme activity appears, reaches its peak, and declines at approximately the same rate as do the other morphological changes produced by estrogen, such as the heightening of the uterine epithelium and the stromal edema (Hooker 1945). It is also evident from the animals receiving prolonged treatment that the enzyme is maintained in the uterine tissues during continued estrogen stimulation. However, further data are needed before we can elucidate the mechanism by which estrogen mobilizes uterine phosphatase.

The function of alkaline phosphatase in the metabolism of the uterus is undetermined. It is possible, however, to compare the potentialities of the enzyme, as demonstrated in other tissues, with the biochemical processes initiated in the uterus by estrogen.

The indispensable role played by phosphate in so many aspects of metabolism has been firmly established by biochemical research and numerous distinct phosphatases have been described. Of these, only the enzyme, or group of enzymes, which are capable of splitting phosphate monoesters under alkaline conditions apply to the present problem. The characteristics of alkaline phosphatase have been investigated in numerous laboratories, the literature being most accessible through the recent excellent review by Moog (1946). Of possible importance in the interpretation of its function in the uterus is the association of alkaline phosphatase with the metabolism of lipids. Although the enzyme may not take part directly in glycogenesis or glycogenolysis, it may be concerned with the transport of intermediate compounds. It may also play an accessory role in expediting reactions which it does not catalyze directly, by supplying necessary phosphate ions from other sources.

Suggestions concerning the possible functions of the uterine phosphatase may be obtained by comparing its potential activities with those which are known to occur in the uterus under the influence of estrogen. The fact that lipids disappear from the uterine glands during estrogen treatment has been demonstrated in the rat by Alden (1946). The increase in alkaline phosphatase in the glands, which we have observed, might well play a part in this phenomenon. Glycogen is also a labile constitutent of the uterus. The administration of estrogen results in the deposition of glycogen in the uterus of the rat (Boettiger 1946), the glycogen level remaining high as long as estrogen is present. Although alkaline phosphatase is not necessary for glycogenesis or glycogenolysis, it may play a part in the transfer of glycogen. Other effects of estrogen which merit attention in this connection are the increase in water content and the growth of the uterus (Astwood 1938, 1939); change in electrolyte pattern (Talbot, Lowry and Astwood 1940); and the rise in oxygen consumption (Aschheim and Gesenius 1933, Khayyal and Scott 1934). Although there is no reason to believe that alkaline phosphatase plays a part in all of these effects of estrogen, it is impossible to either prove its participation or exclude it from consideration until further data are at hand.

In carrying the interpretation of the function of the uterine phosphatase further, it is important to remember that the response of the uterus to estrogen not only conditions it for further preparation for implantation but also alters the mediun through which the spermatozoa must pass. MacLeod (1941) has called attention to the possibility of the importance of uterine glycogen in this connection. The apical location of the uterine phosphatase both in the uterine epithelium and in the glands strengthens the suggestion tiat it may play a part in secretion and thus affect the contents of the uterine lumen.

The high concentration of alkaline phosphatase in the longitudinal layer of the myometrium is almost as unexpected as the pronounced difference between the longitudinal and circular layers in this regard. The effect of estrogen in augmenting the phosphatase content of the circular layer adds another puzzling feature. Although the increased activity of the uterine musculature under the influence of estrogen is well established (Genell 1937, Reynolds 1939), further differential study of the longitudinal and circular muscles is necessary for an interpretation of the function of this enzyme.

SUMMARY

Injection of estrogen into the ovariectomized mouse is fol owed by a marked increase of alkaline phosphatase in the uterine glands and lumenal epithelium and in the circular layer of the myometrium.

The results of the present experiments on the minimal effective dose of estradiol dipropionate indicate that there is a direct ratio between the level of hormone stimulation and the degree of enzyme response. The enzyme activity appears, reaches its peak, and declines at approximately the same rate as do the other morphological changes produced by estrogen. Prolonged treatment with estrogen results in the maintenance of the reaction.

On the basis of these observations and previous data on the effect of estrogen on uterine metabolism, it is suggested that the mobilization of alkaline phosphatase by estrogen is a link in the mechanism by means of which estrogen affects lipid and carbohydrate metabolism in the uterus.

REFERENCES

ALDEN, R. H.: *Anat. Rec.* 94: 445. 1946.
ASCHHEIM, S., AND H. GESENIUS: *Arch. f. Gynäk.* 153: 434. 1933.
ASTWOOD, E. B.: *Endocrinology* 23: 25. 1938.
ASTWOOD, E. B.: *Am. J. Physiol.* 126: 162. 1939.
ATKINSON, W. B., AND H. ELFTMAN: *Proc. Soc. Exper. Biol. & Med.* 62: 148. 1946.
BOETTIGER, E. G.: *J. Cell. & Comp. Physiol.* 27: 9. 1946.
GENELL, S.: Uterin- och vaginalmuskulaturens funktionella uppgifter. Lund, 1937.
GOMORI, G.: *J. Cell. & Comp. Physiol.* 17: 71. 1941.
HOOKER, C. W.: *Anat. Rec.* 93: 333. 1945.
KHAYYAL, M. A., AND C. M. SCOTT: *Quart. J. Exper. Physiol.* 24: 249. 1934.
MACLEOD, J.: *Endocrinology* 29: 583, 1941.
MOOG, F.: *Biol. Rev.* 21: 41. 1946.
REYNOLDS, S. R. M.: Physiology of the Uterus. New York, 1939.
TALBOT, N. B., O. H. LOWRY AND E. B. ASTWOOD: *J. Biol. Chem.* 132: 1. 1940.

EARLY MATING AND PREGNANCY IN THE MONKEY[1]

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THE FUNDAMENTAL similarity of the menstrual cycles of the human and the rhesus monkey (Macaca mulatta) provides an opportunity for collecting exact information on mating in relation to pregnancy by the use of a superb experimental animal under objective observation. The breeding of the rhesus monkey has been carried on in this laboratory under controlled conditions since 1935. Following the basic work of others (Corner 1923, Allen 1926, and Hartman 1932), our results have been such that we have maintained a self-replacing and expanding colony of animals, now including four generations, which has yielded a considerable mass of mating records. A review (van Wagenen 1945) of the first ten years enabled us to state that during the latter half of that time when the mating routine had become stabilized and confined to a discrete number of hours in the cycle, about one-third of the animals, whether nulliparous or multiparous, became pregnant after a single mating. Of course, certain obvious contributive conditions, physiological and social, were met. In the following year the same animals were mated in the same way except for one change in the experimental procedure, viz., the time of mating was displaced six days later in the menstrual cycle. The negative results (van Wagenen 1945a) were further emphasized by allowing the immediate return for a single cycle to the optimal time of mating, after which the expected pregnancies occurred. In the study which forms the subject of this paper the day of mating was advanced five days in the cycle and, after following this plan for the same length of time used in the preceding year of *late* mating, the animals were again allowed to return to the optimal day. There follows a description of this investigation into the early half of the cycle with reference also to the results of late and optimal mating in an effort to evaluate the relative fertility of these parts of the menstrual cycle.

EXPERIMENTAL

In the Obstetric Monkey Colony there is available each year for breeding a group of 20 to 25 female animals. In the main these are mul-

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tiparous monkeys which have been used for breeding in the preceding several years. Since all but the two or three youngest animals have previous breeding histories, there is at hand material for a comparison of reproductive performance for some of these animals as far back as 1937. To avoid seasonal variation mating is begun September first. Since it is impossible to know the actual time of ovulation in a given cycle, it is customary to mate somewhat in advance of the probable time of ovulation. The most favorable day of the menstrual cycle for mating in our experience is the 11th day. This is in harmony with the earlier investigators and was in this laboratory the result of ten successive years of breeding, using the peak days of all the reported ovulation data for this animal. By the term *mating* is meant that the monkey on a prescheduled day of its menstrual cycle is placed in the cage of a male. Adult male animals do not usually live together amicably and so are caged separately; females live two in a cage. Through 1944 the mating period was 48 hours, but in the present experiment the time has been reduced to 24 hours, or overnight mating. It is the interest here to compare the results of breeding in the three years, 1943 to 1945, and the shorter mating period in the last year is believed to have had no detectable influence on the results. A second improvement in routine was made. Heretofore, the first day of the cycle was determined when making morning rounds of the colony at 9 o'clock. However, during the mating months this year an afternoon observation at 5 P.M. led to a greater accuracy in recording day 1 of the cycle. When mating on the 6th day of the menstrual cycle the female was placed in the male's cage at noon and remained until noon of the 7th day. This is an administrative procedure, all cage changes taking place at noon, and insures a regularity of mating plan which is not confused by introducing a new animal into a cage at a time when there would be competition for food, etc.

Mating on the 6th day. Twenty-two adult females made up the breeding group. All of these were multiparous except two. Monkeys 86 and 89, which had matured the preceding year but were considered too young (not ovulating regularly) for mating the previous September. Table 1 identifies the animals and indicates the distribution of the 6th-day matings throughout the four months, September to December inclusive. There were in all sixty-nine matings for the twenty-two animals, more than an average of three matings each. Three pregnancies resulted, 4.3%. The nineteen animals which were not pregnant at the end of the year were then mated on the 11th day of their first cycle in January, when six of the nineteen became pregnant, 31.6%.

An analysis of the menstrual histories of the three animals which were impregnated at early mating appears in Fig. 1. All three animals had given birth to full term infants in the preceding spring or summer. These histories are of interest because one seeks to know the type of

cycle run by an animal which is able to conceive early in the cycle; however, because of the postgestational influence on the cycles of these three animals during September and October, there remains but a single cycle each for Monkeys 00 and 01 preceding the current gestation from which to draw any inference. When Monkey 00 was mated after the early September bleeding it was expected that she

TABLE 1

1945 Monkey (no.)	6th-day mating					11th-day mating Jan.	
	Sept.	Oct.	Nov.	Dec.			
72	m	m	m			m	
92	m	m	m	m		m	
17						m	pregnant
96	m	m	m	m		m	
16			m	m		m	pregnant
22		m	m	m		m	
42	m	m	m	m		m	
48		m	m	m		m	pregnant
70	m	m	m	m		m	
82	m	m	m	m		m	
85	m	m	m	m		m	pregnant
97	m	m	m	m	pregnant		
00	m		m	m	pregnant		
01	m		m	m	pregnant		
07	m	m		m		m	
18		m	m	m		m	
20	m		m	m		m	pregnant
27	m	m	m	m		m	
63		m	m	m		m	
67	m		m	m		m	pregannt
86		m	m	m		m	
89			m	m		m	
Total number of matings and pregnancies				69	3 4.3%	19	6 31.6%

had entered upon ovulating cycles of normal length, since her history during the Spring and Summer had been a post gestational amenorrhoea of 42 days followed by cycles of 34, 29, and 30 days, but one 51-day cycle intervened. The November cycle of 28 days struck the

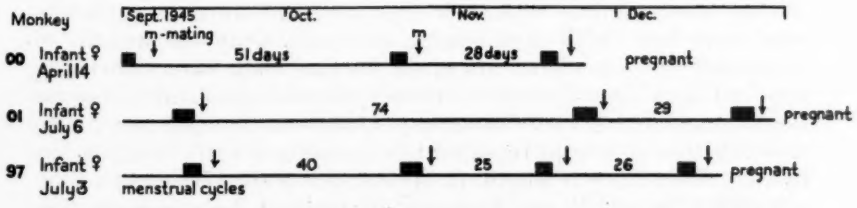

FIG. 1. Menstrual and mating histories of the three monkeys which became pregnant after mating on the 6th day of the menstrual cycle. The amenorrhoeic periods were postgestational irregularities, two animals M.m. 01 and M.m. 97 having given birth to female infants less than two months previously.

modal length. The September matings of Monkeys 01 and 97 were were within 60 days of parturition and were most likely placed at times when the ovaries had not recovered fully their nonpregnant functioning. This is seen in retrospect when an amenorrhoeic period of 74 days and a long cycle of 40 days occurred. A single cycle of 29 days in November suggests normal cycle length, having been resumed

TABLE 2. COMPARISON OF MENSTRUAL CYCLE LENGTHS DURING LATE (17TH-DAY) AND EARLY (6TH-DAY) MATING. FERTILITY RECORD IN THE PREVIOUS YEAR OF OPTIMAL (11TH-DAY) MATING INDICATED

Monkey (no.)	Mating on 17th day (1944)		Mating on 6th day (1945)		Mating on 11th day (1943)	
	Nov. (days)	Dec. (days)	Nov. (days)	Dec. (days)	Nov. (days)	Dec. (days)
72	29	27	31	25	pregnant	
92	29	28	29	29	pregnant	
17	28	28	not mated		pregnant	
96	27	27	27	28	29	27
16	26	27	28	26	pregnant	
22	30	28	30	29	pregnant	
42	27	33	35	26	pregnant	
48	29	29	31	28	pregnant	
70	26	29	27	31	29	30
82	25	23	26	24	pregnant	
85	27	26	26	26	pregnant	
97	26	26	26	pregnant	pregnant	
00	48	pregnant	28	pregnant	pregnant	
01	28	25	29	pregnant	pregnant	
07	28	30		31	32	24
18	32	30	28	30	pregnant	
20	28	25	27	28	pregnant	
27	29	28	29	29	pregnant	
63	24	26	28	26	young	
67	29	30	27	30	young	
86	young		26	24	young	
89	young		31	29	young	
Average	28.2 days		28.1 days			

by Monkey 01, while Monkey 97 is the only one of the three which shows slightly shortened cycles of 25 and 26 days.

Table 2 gives the cycle lengths of all the animals employed in the 6th-day mating group with their cycle lengths during the preceding year when late (17th-day) mating was used. Only the months of November and December are given because they were sufficiently removed from the influence of summer amenorrhoeas and postgestational irregularities. These two groups of data are brought together to show whether or not the repeated late breeding or early breeding has had any effect on cycle length. In the instance of the 17th-day mating, a modification would not be expected, perhaps, because ovulation and the other hormonal processes which bear a causal relationship to the precipitation of menstruation are completed or have passed through the formative stage. By the 17th-day ovulation presumably

has taken place and the ovum is no longer capable of fertilization in the monkey with a 28-day cycle. The single pregnancy which occurred in this group of animals followed two 48-day cycles and, whatever the cause of this deviation from the norm of the year before, it appears that the 17th-day mating coincided well enough with ovulation in the December cycle to have brought about pregnancy.

When mating takes place on the 6th day, however, there is the possibility that the developing follicle which is destined to mature in that particular cycle might be susceptible to the exogenous stimulation of mating. However, if that were so and the maturing processes were hastened to bring about an earlier ovulation, a resultant shorten-

TABLE 3. COMPARISON OF PREGNANCY LENGTH AFTER 6TH- AND 11TH-DAY MATING

Monkey (no.)	6th-day mating		11th-day mating (two preceding pregnancies)	
		Correction of five days		
00	174	169	167	167
01	172	167	165	167
97	168	163	163	167

ing of cycle length would be expected. Sixth day mating is fully five days short of the optimal mating day and establishes an interval which should be reflected in cycle length if any modifying influence had been introduced. Comparison of cycle lengths in Table 2 gives no evidence of shortened cycles in the animals which were repeatedly mated on day 6, the average cycle length being 28.1 days, differing little from the average length of the cycle (28.2 days) in the previous year when mating for these same animals took place on the 17th day. The far column in Table 2 shows the physiological state of these same animals at a similar time in 1943, when only the optimal day for breeding was used. Not all the pregnancies shown for that year followed a single mating, but the usual one-third had so resulted.

One comparison remains to be made. When animals are mated on the eleventh day of the cycle for 48 hours, day 13 is used as the day of conception in estimating length of gestation. With this form of computation the modal length of pregnancy is 168 days, or six times the modal length of the menstrual cycle. In Table 3 the gestation lengths for the three animals which became pregnant after the 6th-day mating are given in the first column, estimated with the 8th-day used as the day of conception. These values seem somewhat high until the correction of five days is applied, when they compare favorably with the data from two preceding pregnancies. All these values lie within the normal limits, but there is here a slight suggestion that, since we can find no evidence for early ovulation, perhaps sperm viability within the genital tract is longer than has been supposed.

DISCUSSION

Since the viability of the free ovum is considerably shorter than that of the sperm, it is the sperm which determine the duration of the interval which can elapse before fertilization and after cohabitation. There appears to be no specific information on the life of the rhesus sperm in the female genital tract, but two or three days is accepted by Hartman. During a 6th-day mating the sperm may be deposited as late as midday on the 7th. Theoretically then, if an ovulation takes place on day 8, 9, or 10, conception could follow. Hartman has reported a single ovulation on day 9 as indicated by an isolated coitus which resulted in pregnancy, and also two ovulations on day 8 and five on day 9, which he determined by rectal palpation. Upon analysis it is not extraordinary then that in the records being considered here three of 22 animals conceived after a 6th-day mating under the regime of this experiment. Two of these animals were running cycles of normal length and the third only slightly shortened cycles, 25 and 26 days.

The question of induced (provoked) ovulation is a very old one. Statistical findings, which failed to allocate ovulation in the human to any one part of the cycle, needed some such theory; i.e., that the ripening of the follicle and ovulation can be brought about by coitus. Conception throughout the cycle was thus explained by the older German gynecologists. There has never been any evidence to show that this is true and, although the data given here were examined for such an influence, apparently the cycle of the monkey cannot be modified by an early sex influence.

This empirical approach to the study of the comparative fertility of selected days in the cycle is an effort to present carefully controlled data of a somewhat different type and perhaps more comparable to human treatment of the known facts concerning ovulation and the cycle. That ovulation, in the normal menstrual cycle having a modal length of twenty-eight days, occurs in the middle of the cycle has been convincingly shown by others. But the sterile parts of the cycle are of unequal value when the days of the cycle as they recede from midpoint are evaluated for fertility. While sensitive to the fact that in numbers the data here presented are not rich, nevertheless it is believed that the difference in the results of mating on the 6th and the 17th days of the cycle represent a real discrepancy in fertility values.

Three recent papers have appeared on the time of ovulation in women (Farris 1946, Sevitt 1946) and monkeys (Farris 1946a) which deal with the early half of the menstrual cycle. Farris, using a biological test, hyperemia of rat ovaries following injection of urine from selected days of the cycle, found ovulation falls within the accepted days, 8 to 19, of the cycle normal in length. Sevitt, interpreting the age of endometrial samples showing secretory activity to the fine point of a single day, believes that ovulation can occur on any day of the first half of the cycle.

SUMMARY

This investigation into the early half of the menstrual cycle is part of a study of the comparative fertility of selected days in the cycle. The empirical approach is comparable to the broad human treatment of the known facts concerning ovulation and the cycle.

The normal reproductive achievement of the 22 rhesus monkeys used was well known; i.e., over a period of several years, when mated on the 11th day of the menstrual cycle, 30% of the animals became pregnant following a single mating. In this study these animals were mated on the 6th day instead of the 11th, and after 69 matings three animals were pregnant, 4.3%. The remaining nineteen animals, which failed to become pregnant during the 6th-day mating period, were then mated in the single cycle immediately following on the 11th day, after which six pregnancies resulted, 31.6%.

Since cycle lengths were not influenced by repeated early mating, there was no evidence for induced ovulation.

REFERENCES

ALLEN, E.: *Proc. Soc. Exper. Biol. & Med.* 23: 381. 1926.
CORNER, G. W.: *Contrib. to Embryol.* 15: 75. 1923.
FARRIS, E. J.: *Am. J. Obst. & Gynec.* 52: 14. 1946.
FARRIS, E. J.: (a) *Anat. Rec.* 95: 337. 1946.
HARTMAN, C. G.: *Contrib. to Embryol.* 23: 1. 1932.
SEVITT, S.: *Lancet* 251: 448. 1946.
VAN WAGENEN, G.: *Yale J. Biol. & Med.* 17: 745. 1945.
VAN WAGENEN, G.: *Endocrinology* 37: 307. 1945.

THE "SPARING" EFFECT OF PROGESTERONE ON THE HEPATIC INACTIVATION OF α-ESTRADIOL

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PINCUS and Zahl (1937) demonstrated that progesterone exerts a "sparing" effect on the destruction of estrogens in rabbits. This work has been confirmed and greatly extended to man by the studies of Smith and Smith (1946). Their studies would also indicate that at least part of the defect in the toxemias of pregnancy is due to progesterone deficiency. White (1943) has applied these findings to the toxemias of pregnant diabetic patients in whom progesterone therapy has given striking results. The decreased infant mortality in such cases is particularly impressive. The Smiths have continued their studies and the effects of progesterone upon estrogen metabolism now form one of the basic facts upon which their current theory of the causation and purpose of menstruation rests. They found that "Progesterone enhances the metabolic conversion of estradiol (the primary ovarian estrogen) to estrone and on to estriol, thus decreasing the oxidative inactivation that appears to take place in the reversible estradiol to to estrone reaction."

On the other hand, Heard, Bauld & Hoffman (1941), employing the isolation of crystalline urinary end products of estrogen metabolism in rabbits, were unable to demonstrate any discernable effect of progesterone administration. Furthermore, Heller and Heller (1943) have demonstrated that livers from pregnant animals inactivate estrogens *in vitro* to the same extent as do livers from nonpregnant animals.

MATERIAL AND METHODS

Two strains of rats of moderately different ability to inactivate estrogens, the inbred Fischer and Copenhagen strains, were employed (Segaloff and Dunning (in press)). The methods were the same as reported previously in our studies on estrogen metabolism (Segaloff, 1943, 1946; Segaloff and Dunning (in press)). The amount of α-estradiol for each assay was injected in 0.05 cc. of 5% benzyl alcohol in sesame oil in spayed female rats which had been primed fourteen days previously with 50 μg. of estrone in peanut oil. The animals treated with progesterone were given subcutaneous injections of 0.25 mg. in 0.1 cc. of sesame oil twice daily starting the day prior to the injection of estradiol and continuing until the completion of the assay period.

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RESULTS

The results calculated from the amounts of α-estradiol required for 50% vaginal estrus response are presented in Figure I. Each figure represents the data on an assay employing 20 animals at each level of α-estradiol. Additional greater and lesser amounts of α-estradiol were employed and the responses were consistant with dosage. As can be seen from the circles, the administration of progesterone raised the threshold for vaginal estrus reducing the effectiveness of the subcutaneously administered α-estradiol approximately one half. In contrast to this is the result when the α-estradiol was administered intrasplenically where the progesterone raised the threshold only slightly. This has the effect of decreasing the ratio of the amount required intrasplenically to that required subcutaneously. Thus, progesterone halved the inactivation of α-estradiol in both strains studied.

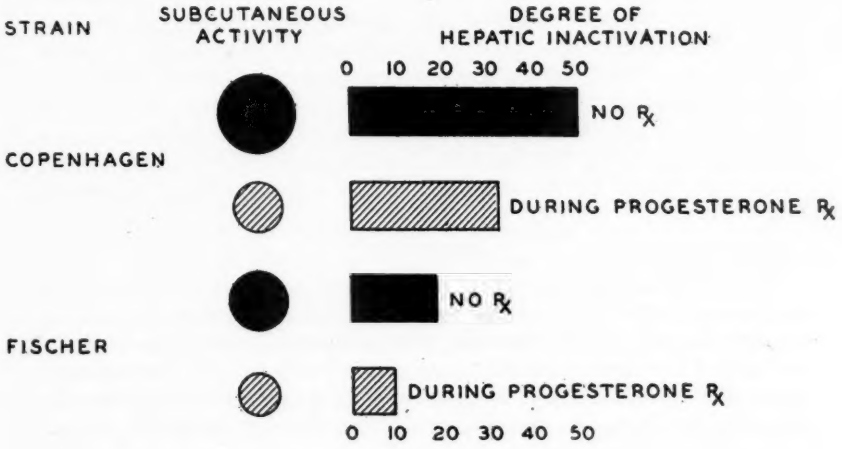

FIG. 1. The effect of progesterone on the inactivation of α-estradiol.

DISCUSSION

The action of progesterone on the hepatic inactivation of estrogens is indeed a complex one. The evaluation of its action is made more difficult by the fact that there appears to be a double action of progesterone. Each of these actions is opposite in result when measured by the method of vaginal smears.

Progesterone decreased the ratio (subcutaneous to intrasplenic) between the amount of α-estradiol required for the vaginal estrus in 50% of the animals. This is a fact which we interpret as indicating a

decrease in the hepatic inactivation of the α-estradiol. A similar but more marked decrease in this ratio occurs when the greater part of the liver is removed surgically (Segaloff, 1946). However, when the liver is excised, there is another difference. The decrease in inactivation of estrogen apparently makes a greater amount available to act upon the end organs and the amount of subcutaneously administered α-estradiol required to produce estrus in 50% of the animals is consequently reduced. To say this in other words, the vaginal response to a given amount of subcutaneously injected estrogen is increased by the removal of a greater part of the liver. On the other hand, the administration of progesterone increases the amount of subcutaneously administered α-estradiol required for vaginal estrus in 50% of the animals. Thus, despite the fact that the decreased hepatic inactivation would lead us to expect a greater response to subcutaneously administered estrogen the opposite effect is found during progesterone therapy. This can be explained if we assume that in addition to its action in decreasing the hepatic inactivation of α-estradiol progesterone also decreases the sensitivity of the vaginal mucosa to α-estradiol.

SUMMARY

In studies of the inactivation *in vivo* of α-estradiol by the rat's liver, it was found that administration of 0.5 mg. of progesterone daily reduces the amount inactivated. The peripheral effectiveness of the α-estradiol for vaginal cornification is decreased by the simultaneous administration of 0.5 mg. of progesterone daily. These findings are discussed in relation to the decreased hepatic inactivation of α-estradiol produced by partial hepatectomy.

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REFERENCES

HEARD, R. D. H., W. S. BAULD AND M. M. HOFFMAN: *J. Biol. Chem.* 141: 709. 1941.
HELLER, C. G., AND E. J. HELLER: *Endocrinology* 32: 64. 1943.
PINCUS, G., AND P. A. ZAHL: *J. Gen. Physiol.* 20: 879. 1937.
SEGALOFF, A.: *Endocrinology* 33: 209. 1943.
SEGALOFF, A.: *Endocrinology* 38: 212. 1946.
SEGALOFF, A., AND W. F. DUNNING: *Endocrinology.* 39: 289. 1946.
SMITH, G. VAN S., AND O. W. SMITH: *J. Clin. Endocrinol.* 6: 483. 1946.
WHITE, P., AND H. HUNT: *J. Clin. Endocrinol.* 3: 500. 1943.

THE METABOLIC SIGNIFICANCE OF PROTEIN-BOUND IODINE OF PLASMA: A STUDY OF ITS CONCENTRATION UNDER VARIOUS CONDITIONS AND OF ITS RATE OF FORMATION AS MEASURED WITH RADIOACTIVE IODINE[1]

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THE FRACTION of plasma iodine designated "protein-bound" is the iodine that is precipitated from plasma along with its proteins by such agents as tungstic acid, zinc hydroxide, or acetic acid in the presence of heat and in addition cannot be freed from these proteins by simple washing.[2] Although the chemical identity of this fraction is as yet poorly understood, its concentration in plasma has gained wide use as an indicator of thyroid activity in man. (Salter *et al.*, 1941; Man *et al.*, 1942; Lowenstein *et al.*, 1944; Talbot *et al.*, 1944; Chaney, unpublished observations).

In the present investigation, an attempt has been made to determine the metabolic significance of plasma protein-bound iodine. Its concentration in plasma was studied in rats shown to be *completely* deprived of thyroid tissue and in rats and guinea pigs in which hyperthyroidism had been induced. The use of radioactive iodine (I^{131}) as a labeling agent also made possible a study of the rate of formation of protein-bound iodine in both states of thyroid activity.

EXPERIMENTAL

The rats used in this study were males of the Long-Evans strain weighing from 200 to 300 gm. After weaning they were fed a diet that contained 0.3 micrograms of iodine per gm. and had the following composition: 68.5 per cent whole wheat, 5 per cent casein, 10 per cent fish meal, 5 per cent fish oil, 10 per cent alfalfa, and 1.5 per cent sodium chloride.

The guinea pigs used were 400–500 gm. males which during the experimental period were fed a diet of pellets (a commercial preparation that contained 0.35 micrograms of iodine per gram), barley, and lettuce.

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[2] Dialysis (Silver and Tysen, 1945) and fractionation with organic solvents (Davison *et al.*, 1942; Perkin and Hurxthal, 1939) have also been used to separate so called "hormonal" from inorganic iodine of plasma.

Preparation of Animals—Rats were thyroidectomized while under nembutal anesthesia. The completeness of thyroidectomy was tested in most cases by the radioautograph procedure described elsewhere (Reinhardt, 1942). The tissues examined by this procedure for iodine-concentrating capacity included the base of the tongue, trachea, oesophagus, heart, lungs, great vessels and associated muscles of the neck region. Radioautograms were obtained by placing all neck and chest tissues between 2 non-screen x-ray films and permitting the exposure to last for 24–48 hours. The films were developed and the relative areas of blackened film compared.

The thyroid glands of rats and guinea pigs were made hyperactive by the

TABLE 1. RATE OF DECREASE OF PLASMA PROTEIN-BOUND IODINE AFTER THYROIDECTOMY

Experiment 1			Experiment 2		
Interval after operation	Weight of rat	Protein-bound iodine of plasma	Interval after operation	Weight of rat	Protein-bound iodine of plasma
	gm.	micrograms per cent		gm.	micrograms per cent
Control*	280	3.0	Control†	305	3.7
Control*	262	3.6	Control†	265	3.5
Control*	267	3.8	Control†	289	4.5
4 hours	263	2.9	5 hours	317	3.0
4 hours	260	2.8	5 hours	319	3.0
8 hours	246	2.5	8 hours	317	2.3
8 hours	280	2.5			
8 hours	289	3.0	9 hours	281	2.1
12 hours	263	2.0			
12 hours	257	2.6			

* These controls were subjected to no operative procedures.
† These controls were subjected to a sham operation which consisted of exposing the thyroids under nembutal anesthesia.

subcutaneous injection of a thyrotropic hormone preparation containing 4 guinea-pig units per mg.[3] The preparation was dissolved in saline and administered in doses of 10 mg. twice daily in the case of rats, and 2 mg. once daily in the case of the guinea pigs.

Iodine Determinations—Blood was taken directly from the heart while the animals were anesthetized with nembutal. Three cc. samples of plasma were used for the determination of protein-bound iodine; the procedure employed has been described elsewhere (Taurog and Chaikoff, 1946a). The method by which total and thyroxine iodine of the thyroid gland was measured has also been described (Taurog and Chaikoff, 1946b).

Experiments with Radioactive Iodine—Radioactive inorganic iodine (I^{131})

[3] Kindly furnished by Dr. D. A. McGinty of Parke, Davis and Co. The unit is defined as the amount which, when injected subcutaneously once daily for 4 days into 180–200 gm. guinea pigs, produces on the fifth day minimal but definite hyperplasia of the thyroid in all of six animals.

containing no carrier was used in this investigation. Each animal was injected intraperitoneally with 10–20 microcuries of I^{131} and sacrificed at specified intervals thereafter. Plasma proteins were precipitated by adding one cc. of the plasma to 10 cc. of 10-per cent trichloroacetic acid. It was found desirable to use trichloroacetic acid for the precipitation of plasma proteins since the precipitate formed is readily dissolved by dilute alkali. The precipitate was washed twice, each time with 5 cc. of 10 per cent trichloroacetic acid. It was then dissolved with the aid of a few drops of 2 N NaOH. The radioactivity was determined by means of a scale-of-8 Geiger-Muller counter.

TABLE 2. PLASMA PROTEIN-BOUND IODINE 3 AND 16 DAYS AFTER THYROIDECTOMY

Controls	3 days after thyroidectomy		16 days after thyroidectomy	
Plasma iodine	Plasma iodine	Completeness of thyroidectomy as shown by radioautogram	Plasma iodine	Completeness of thyroidectomy as shown by radioautogram
micrograms per cent	micrograms per cent	*	micrograms per cent	*
2.8	1.6	complete	1.3	complete
3.1	1.2	complete	1.2	complete
3.1	0.9	complete	1.1	incomplete
3.6	0.5	complete	1.0	incomplete
3.6	1.1	complete	1.6	incomplete
	0.8	incomplete	0.5	incomplete
	1.5	incomplete	1.5	grossly incomplete
	1.2	incomplete	1.0	grossly incomplete
	0.6	incomplete	0.8	grossly incomplete
	0.9	incomplete		
	0.8	grossly incomplete		

* Thyroidectomy was judged to be complete when the radioautogram showed no area of blackening. It was judged to be incomplete when the radioautogram showed areas of blackening up to 5 per cent of the controls. The grossly incomplete thyroidectomy was that in which blackening up to 25 per cent of the controls appeared.

I. EXPERIMENTS ON THYROIDECTOMIZED RATS

Rate of Fall of Plasma Protein-bound Iodine after Thyroidectomy—In Table 1 are recorded the results of 2 experiments in which protein-bound iodine of plasma was measured at intervals between 4 and 12 hours after thyroidectomy. In order to increase the relative accuracy of the results each experiment was carried out on a uniform group of rats and the iodine analyses done at the same time.

A significant decrease in the level of protein-bound iodine of plasma was evident as early as 4 hours after removal of the thyroids. In 12 hours the decrease amounted to about 30 per cent.

In the experiments of Table 2 the levels of protein-bound iodine were determined at much later intervals after thyroidectomy, namely 3 and 16 days. The mean values for plasma protein-bound iodine at the third and sixteenth day were 1.1 and 1.0 micrograms per cent respectively, as compared with an average control value of 3.2 micro-

grams per cent. Thus by the third day the level of this constituent had decreased to its lowest value. The *absolute* significance of these low values, however, is difficult to assess since they depend upon colorimetric readings which differ only slightly from those given by the reagent-blanks. Whether there exists in the completely thyroidecto-

TABLE 3. FORMATION OF PLASMA PROTEIN-BOUND I^{131} BY THYROIDECTOMIZED RATS

Expt.	Treatment	Interval when animals sacrificed		Plasma			Completeness of thyroidectomy as shown by radioautogram
		After operative procedure	After I^{131} injection	Total I^{131} as per cent injected dose	Protein-bound I^{131} as per cent of injected dose	Protein-bound I^{131} / Total I^{131}	
		hours	hours	per 10 cc. plasma	per 10 cc. plasma	×100	
1	Sham Operated Control	60–72	13	1.1	0.54	49	
	Sham Operated Control	60–72	13	0.96	0.65	68	
	Thyroidectomized	60–72	13	3.0	0.16	5.3	complete
	Thyroidectomized	60–72	13	2.9	0.17	5.9	complete
	Thyroidectomized	60–72	13	2.8	0.22	7.9	complete
	Thyroidectomized	60–72	13	3.6	0.22	6.1	incomplete
	Thyroidectomized	60–72	13	5.3	0.33	6.2	incomplete
2	Unoperated Control		17	3.0	2.2	73	
	Unoperated Control		17	1.5	1.1	73	
	Unoperated Control		17	2.8	2.0	72	
	Thyroidectomized	75–90	17	2.0	0.12	6.0	complete
	Thyroidectomized	75–90	17	2.3	0.15	6.5	complete
	Thyroidectomized	75–90	17	1.8	0.11	6.1	complete
	Thyroidectomized	75–90	17	3.0	0.14	4.7	incomplete
	Thyroidectomized	75–90	17	2.2	0.16	7.3	incomplete
	Thyroidectomized	75–90	17	2.7	0.14	5.2	incomplete
	Thyroidectomized	75–90	17	1.5	0.055	3.7	incomplete
	Thyroidectomized	75–90	17	2.1	0.14	6.7	incomplete
	Thyroidectomized	75–90	17	4.0	0.15	3.8	incomplete
	Thyroidectomized	75–90	17	1.6	0.087	5.4	grossly incomplete
	Thyroidectomized	75–90	17	2.3	0.28	12.2	grossly incomplete
3	Sham Operated Control	64–72	25	1.1	0.94	85	
	Sham Operated Control	64–72	25	1.3	1.1	88	
	Thyroidectomized	64–72	25	1.2	1.1	94	
	Thyroidectomized	64–72	25	2.0	1.8	91	
	Thyroidectomized	64–72	25	2.4	0.18	7.5	complete
	Thyroidectomized	64–72	25	0.74	0.075	10	complete
	Thyroidectomized	64–72	25	2.0	0.15	7.5	complete
	Thyroidectomized	64–72	25	1.4	0.083	5.9	complete
	Thyroidectomized	64–72	25	1.8	0.092	5.1	complete
	Thyroidectomized	64–72	25	1.9	0.083	4.4	complete
	Thyroidectomized	64–72	25	1.5	0.15	10	incomplete
	Thyroidectomized	64–72	25	1.4	0.14	10	incomplete
	Thyroidectomized	64–72	25	2.3	0.12	5.2	incomplete
	Thyroidectomized	64–72	25	1.6	0.083	5.2	incomplete
	Thyroidectomized	64–72	25	1.6	0.36	22	grossly incomplete

mized rat a small amount of protein-bound iodine must therefore remain an open question.

It is of interest to note that the concentration of protein-bound iodine of plasma in rats in which, as judged by radioautograms, an appreciable amount of thyroid tissue still existed was of the same magnitude as that observed in rats shown to be completely thyroidectomized by the same procedure. Evidently this residual thyroid tissue contributes very little to the protein-bound iodine of plasma.

Rate of Formation of Plasma Protein-bound Iodine (as measured with radioactive iodine) in Thyroidectomized Rat—The rate at which injected radioactive inorganic iodine is converted to protein-bound iodine of plasma is shown in Table 3. After its introduction into *normal* rats, radioactive inorganic iodine makes its appearance rapidly in the

protein-bound iodine fraction of plasma. In 13 hours, 50 per cent of plasma's I^{131} was found to be protein-bound, in 24 hours 90 per cent.

In the *completely thyroidectomized* rat, on the other hand, only 5–10 per cent of plasma's I^{131} was protein-bound as late as 25 hours after the injection. In only 2 of the rats subjected to thyroidectomy was the protein-bound I^{131} higher than 10 per cent of the total I^{131}, but it should be noted, both animals contained appreciable fragments of residual thyroid tissue

The method employed here of washing the precipitated plasma proteins did not completely remove added radioactive inorganic iodine. When rats were injected with I^{131} and their plasma taken 5 minutes later, several per cent of the radioactivity in the plasma remained with the precipitated plasma proteins after 2 washings. Thus, even the small amounts of I^{131} recorded in Table 3 as protein-bound in the thyroidectomized rats are probably too high.

The data presented in Table 3 indicate that the formation of protein-bound iodine is greatly diminished in completely or nearly completely thyroidectomized rats.

II. EXPERIMENTS ON RATS AND GUINEA PIGS WHOSE THYROIDS WERE MADE HYPERACTIVE BY INJECTIONS OF THYROTROPIC HORMONE

The Protein-bound Iodine of Plasma of Rats and Guinea Pigs Injected with Thyrotropic Hormone—The effect of injections of thyrotropic hormone on the level of protein-bound iodine of plasma is shown in Table 4. The elevation of plasma iodine in rats that had received injections for 4 days (Group B) was not nearly so marked as in those that were injected for a single day (Group C). In the former case the elevation was definite but small in extent; in the latter the average increase was 70 per cent. A more striking increase was observed in the case of guinea pigs that had received injections for 4 days. The protein-bound iodine level in the latter rose from a mean control value of 2.2 micrograms per cent to one of 5.8, an increase of 165 per cent.

A reduction of the iodine content of the thyroids of animals that received thyrotropic hormone injections is also shown in Table 4.

Rate of Formation of Plasma Protein-bound Iodine (as measured with radioactive iodine) in Rats and Guinea Pigs Injected with Thyrotropic Hormone—The appearance of injected radioactive inorganic iodine in protein-bound iodine of plasma of hyperthyroid animals is shown in Table 5. A striking increase above control values was observed as early as 3 hours after I^{131} injection. At this interval the percentage of the injected I^{131} found in the protein-bound iodine fraction per 10 cc. of plasma varied from 0.73 to 1.3 in hyperthyroid rats and from 0.17 to 0.29 in the control rats. In the former, 39–73 per cent of plasma's I^{131} was protein-bound, whereas in the controls only 4.1–9.2

per cent was so bound. Large differences between the 2 groups were also evident 6 hours after the injection of the radioactive isotope; at this interval 11–32 per cent of plasma's I^{131} was protein-bound in the control rats as compared with 82–87 per cent in the hyperthyroid rats. A pronounced increase in the rate of incorporation of injected radio-

TABLE 4. EFFECT OF THYROTROPIC HORMONE ON PROTEIN-BOUND IODINE OF PLASMA

Animal	Group	Weight	Treatment	Plasma protein-bound iodine	Thyroid		
					Weight	Total iodine	Thyroxine iodine
		gm.		micrograms per cent	mg.	micrograms	micrograms
Rats	A	246 240	Control Control	3.2 3.2	33 28	6.1*	
Rats	B	218 240 229 238 224 219	10 mg. thyrotropic hormone injected twice daily for 4 days, and once on the 5th day 2 hours before animals were sacrificed.	3.8 3.3 4.7 3.7 4.5 3.9	50 74 58 78 58 50	1.7*	
Rats	C	260 257 279 270	10 mg. thyrotropic hormone injected twice on the first day, and once on the 2nd day 1.5 hours before animals were sacrificed.	4.9 6.2 5.3 5.7	34 45 43 43	4.3*	
Guinea Pigs	A	478 460 495 541	Control Control Control Control	2.2 2.0 2.0 2.5	75 56 68 68	37.0 25.1 31.4 20.8	9.0 5.8 8.2 4.3
Guinea Pigs	B	433 428 424 426	2 mg. thyrotropic hormone injected once daily for 3 days, and twice on 4th day. Animals sacrificed morning of 5th day.	6.1 6.1 4.4 6.8	113 147 106 120	19.0 11.2 5.5 11.0	4.2 2.3 1.2 2.4

* Thyroids of all rats of each group were pooled for analysis.

active iodine into plasma protein-bound iodine was also observed in the guinea pigs possessing hyperactive thyroids.

DISCUSSION

With respect to both its concentration and its rate of formation, protein-bound iodine of plasma responded to experimentally induced changes in thyroid activity in a manner that would be expected in the case of a circulating thyroid hormone. Thus the level of protein-bound iodine of plasma was elevated by the injection of thyrotropic hormone and was depressed by the removal of the thyroids. Furthermore the rate of appearance of injected radioactive inorganic iodine in the pro-

tein fraction of plasma was greatly diminished by thyroidectomy and greatly augmented by injection of thyrotropic hormone. This rate of appearance may prove useful as an index of thyroid activity.

The rate of decrease in the level of plasma protein-bound iodine is more rapid after thyroidectomy than after hypophysectomy. After excision of the pituitary gland the plasma protein-bound iodine level decreased about 20 per cent in 2 days (Taurog, Chaikoff and Bennett,

TABLE 5. FORMATION OF PLASMA PROTEIN-BOUND I^{131} BY RATS AND GUINEA PIGS INJECTED WITH THYROTROPIC HORMONE

Animal	Interval after I^{131} injection	Controls				Thyrotropic Hormone*			
		Weight	Weight of thyroid glands	Protein-bound I^{131} as per cent of injected dose	Protein-bound I^{131} / Total I^{131}	Weight	Weight of thyroid glands	Protein-bound I^{131} as per cent of injected dose	Protein-bound I^{131} / Total I^{131}
	hours	gm.	mg.	per 10 cc. plasma	×100	gm.	mg.	per 10 cc. plasma	×100
Rats	3	239	23	0.29	7.3	188	36	2.9	73
	3	194	22.5	0.21	5.2	180	30	1.3	39
	3	207	29	0.17	4.1	211	76	0.73	53
	3	240	33.5	0.26	9.2				
	6	231	31.5	0.44	30	185	39	2.0	82
	6	185	19	0.36	11	230	49	4.1	88
	6	263	33	0.34	27	186	43.5	3.1	87
	6	218	38	0.31	32				
	12	240	33	1.6	95	252	43	1.5	91
	12	235	25.5	1.7	86	191	38	3.3	100
	12	234	22	1.2	80	240	64	1.8	95
	12	219	32	1.3	94	197	34	5.9	89
	24	231	26	1.5	87				
	24	252	27	1.4	88				
	24	201	17	2.0	92				
Guinea Pigs	16	478	75	0.041	7.5	433	113	0.70	77
	16	460	56	0.045	12	428	147	1.4	83
	16	495	68	0.031	12	424	106	1.1	77
	16	541	68	0.064	18	426	120	1.3	81
						336	112	0.99	86

* The rats were injected with 10 mg. of thyrotropic hormone twice daily for 4 days. The 3 hr. and 6 hr. groups also received one injection on the 5th day at the same time that the I^{131} was injected. The guinea pigs were treated as described in Table 4.

1946), whereas a decrease of over 30 per cent was evident as early as 12 hours after removal of the thyroids.

The chemical nature of plasma protein-bound iodine requires further clarification. Previous work indicates that it consists of more than one iodine-containing compound (Wilmanns, 1943). If protein-bound iodine of plasma should prove to be complex in nature, its fractionation may provide a component that is more closely identified with the actual circulating hormone and may be a more sensitive index of thyroid activity than total protein-bound iodine.

SUMMARY

The level of protein-bound iodine in the plasma of rats falls rapidly after thyroidectomy. A noticeable decrease occurred in 4 hours. On the third day the level dropped to its minimum value.

The rate of incorporation of injected radioactive inorganic iodine

into the protein-bound iodine fraction of plasma is depressed in thyroidectomized rats.

The levels of plasma protein-bound iodine are increased in rats and guinea pigs injected with thyrotropic hormone.

The rate of appearance of injected radioactive inorganic iodine in the protein-bound iodine fraction of plasma is greatly augmented in rats and guinea pigs that have received injections of thyrotropic hormone.

It is suggested that the rate of conversion of injected radioactive inorganic iodine into protein-bound iodine of plasma is a useful guide to thyroid activity.

REFERENCES

CHANEY, A. L.: private communication. 1944.

DAVISON, R. A., R. W. ZOLLINGER AND G. M. CURTIS: *J. Lab. & Clin. Med.* 27: 643. 1942.

LOWENSTEIN, B. E., M. BRUGER AND J. W. HINTON: *J. Clin. Endocrinol.* 4: 268. 1944.

MAN, E. B., A. E. SMIRNOW, E. F. GILDEA AND J. P. PETERS: *J. Clin. Investigation* 21: 773. 1942.

PERKIN, H. J., AND L. M. HURXTHAL: *J. Clin. Investigation* 18: 733. 1939.

REINHARDT, W. O.: *Proc. Soc. Exper. Biol. & Med.* 50: 81. 1942.

SALTER, W. T., A. M. BASSET AND T. S. SAPPINGTON: *Am. J. M. Sc.* 202: 527. 1941.

SILVER, S., AND M. C. TYSON: *J. Mt. Sinai Hosp.* 12: 701. 1945.

TALBOT, N. B., A. M. BUTLER, A. H. SALTZMAN AND P. M. RODRIGUEZ: *J. Biol. Chem.* 153: 479. 1944.

TAUROG, A., AND I. L. CHAIKOFF: *J. Biol. Chem.* 163: 313. 1946a.

TAUROG, A., AND I. L. CHAIKOFF: *J. Biol. Chem.* 163: 323. 1946b.

TAUROG, A., I. L. CHAIKOFF AND L. L. BENNETT: *Endocrinology* 38: 122. 1946.

WILMANNS, H.: *Ztschr. f. d. ges. exper. Med.* 112: 1. 1943.

THE INCIDENCE OF DELAYED OVULATIONS FOLLOWING ABDOMINAL OPERATIONS IN THE DOMESTIC HEN

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INTRODUCTION

WE SHOWED recently (Rothchild and Fraps, 1945) that abdominal operations in the domestic hen were capable of inducing follicular atresia and that the incidence of this effect was inversely related to the frequency of ovulation preceding the operation. It was also noted in this report that the oldest preovulatory follicle showed what seemed to be a delayed ovulation in a large percentage of those birds which failed to undergo atresia following operation. This effect has been further investigated and confirmed, and is reported on in detail in the present communication.

METHODS AND MATERIALS

General procedures. The breeds of birds used, their ages, maintenance, operative procedures, and postoperative treatment have been described previously (Rothchild and Fraps, 1945). Six series of operations were performed, five of which involved opening the body cavity, with and without manipulations of the ovary; the sixth series of operations involved lacerations of the breast muscle. All the birds in the five series of abdominal operations will be treated together as one group, since only minor differences existed among them.

All the birds presented here were subjected to operation on the day of ovulation of either the first egg of a clutch (C_1 ovulation, 190 birds), or of the second egg of a clutch (C_2 ovulation, 13 birds). The length of the clutch immediately preceding operation in the group of birds operated on on the day of C_1 ovulation varied from 1 egg to 4 eggs or more; those subjected to operation on the day of a C_2 ovulation had a clutch length immediately preceding operation of not fewer than 4 eggs. The reason for this selection will appear below. The operations were performed between 0.5 and 14 hours after ovulation but in the majority of birds between 4 and 8 hours after ovulation.

Ovulation was determined by digital palpation through the rectum of an oviducal egg formed following operation, or by the finding of such an egg in the oviduct at autopsy, or of yolk fluid (in the absence of atresia) in the body cavity together with a ruptured follicle of appropriate size on the ovary.

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If the yolk fluid was thin, and the ruptured follicle comparatively small, it indicated that ovulatiou occurred on the day preceding autopsy; if the yolk fluid was thick and the ruptured follicle comparatively large, that ovulation occurred on the day of autopsy.

Determination of the incidence of delayed ovulations

1. *General considerations.* In the normal intact chicken, the successive ovulations within a clutch (C_n ovulations) follow the lay of the preceding egg by an interval which rarely exceeds one hour, the usual interval being about 30 minutes in hens laying out clutches of moderate length (Phillips and Warren, 1937). In contrast to this close association between time of lay and C_n ovulations, the ovulation of the first follicle of a clutch (C_1 ovulation) in birds in regular lay, under the light conditions in this laboratory, almost always occurs during the early morning hours (5:00 a.m. to 8:00 a.m.) of the day following that on which the last egg of the preceding clutch was laid, or at an interval of about 18 hours from the time of lay of the preceding egg. In birds in irregular lay, and sometimes even in birds in regular lay, this interval may be lengthened by an additional one or more full days.

2. *Definition of a delayed ovulation.* For the purpose of this study a delayed ovulation is defined as the ovulation of a theoretically scheduled C_n follicle at a time not earlier than the second day following that on which the preceding egg (the oviducal egg present at operation) was ovulated. The interval between ovulation and time of lay of practically all eggs is, in general, zero to a few hours more than 24 hours (0 to 6 hours). Therefore, from what has been said in the preceding paragraph, the expected time for a non-delayed or C_n ovulation in these experiments was on the day following operation. Similarly, all delayed ovulations, as defined here, occurred only on the second day or later following operation. Several birds which were autopsied on the first day after operation without having ovulated, but at a time later than expected for a normal C_n ovulation, were excluded from consideration. Any error involved in using this definition would thus be in the direction of demonstrating a minimum rather than a maximum effect.

3. *Criteria used to distinguish between normal C_1 and delayed ovulations.* In practically every case, the hour of the day on which a delayed ovulation occurred more nearly approximated the hour characteristic of most C_1 ovulations than it did the expected hour of ovulation of the particular follicle showing the delay. The delay was thus not a 24 hour postponement of the time of expected C_n ovulation, but appeared to involve a change in the characteristic behavior of the C_n follicle in the direction of typical C_1 behavior. The normal C_1 ovulations and the experimentally induced delayed ovulations that resembled them therefore could be distinguished from each other only in a quantitative way. This was done by comparing the incidence of C_1 type ovulations after operation with the incidence of these ovulations in normal birds,

in relation to the length of the preceding clutch. The C_1 type ovulation was defined as any ovulation that occurred during the early morning hours (see above) on the second day or later after the preceding ovulation.

The occurrence of a C_1 type ovulation in the oldest preovulatory follicle after operation meant that the oviducal egg present at the time of operation became the terminal member of the clutch to which it belonged, regardless of whether the next follicle was due to ovulate normally as a C_1 type follicle or was induced to do so as a result of operation. Thus, in the case of those birds subjected to operation on the day of ovulation of the first follicle of a clutch, the subsequent occurrence of a C_1 type ovulation meant that the oviducal egg became the only member of a 1 egg clutch;[1] in the case of those birds subjected to operation on the day of ovulation of the second follicle of a clutch, similarly, it meant that this oviducal egg became the terminal member of a 2 egg clutch.[1] The basis for comparison with the normal incidence of C_1 type ovulations was thus the incidence of 1 and 2 egg clutches, following clutches of varying length. This was determined as follows.

From the daily lay records of each of 413 normal birds, a series of 5 successive clutches was chosen, all eggs being laid during the same months as those in which operations were performed. Only those birds were selected which showed no more than 2 day intervals between any successive pair of clutches in the five. A table was then drawn up, with 4 columns numbered 1, 2, 3, and 4 or more and 5 rows numbered, 0, 1, 2, 3, and 4 or more. Each column served to designate the length of the preceding clutch, and each row the length of the following clutch, the 0 row being used to designate a 2 day interval following a clutch. Each of the 5 clutches except the last was then in turn considered as a preceding clutch and under the appropriate column the length of the following clutch was indicated by a check in the appropriate row. For instance, a particular bird showed the following sequence of clutches: 2–3–2–2–1; under the column for preceding clutches of 2 eggs, one case was noted where the following clutch was 3 eggs, one case where it was 2 eggs and one case where it was 1 egg, while under the column for preceding clutches of 3 eggs, one case was recorded where the following clutch was 2 eggs.

Thus the frequency of occurrence of clutches of length 1 to 4 eggs or more was determined in relation to the length of the preceding clutch. We are concerned here, however, only with the incidence in normal birds of 1 and 2 egg clutches, or, with what amounts to the same thing for this particular study, the incidence of C_1 type ovulations following the lay of the first or the second egg of a clutch in relation to the length of the preceding clutch.

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[1] All those birds in which atresia of the oldest preovulatory follicle took place were, of course, excluded from consideration.

The birds subjected to operation on the day of ovulation of the second egg of a clutch were limited to those having a clutch length immediately preceding operation of not fewer than 4 eggs. This procedure was followed because in normal birds the probabilities for clutches of no more than 2 eggs to follow clutches of fewer than 4 eggs were relatively so great that large numbers of birds would have been required to demonstrate any alteration in these probabilities as a result of operation.

4. *Calculation of the incidence of delayed ovulations.* A. *In the birds not showing atresia after operation.* In each group of birds classified according to the length of the last clutch before operation, the number of birds not showing atresia and which were expected to ovulate the

TABLE 1. AVERAGE OVULATION FREQUENCIES OF THE GROUPS OF BIRDS SUBJECTED TO OPERATION WHOSE LAST CLUTCHES BEFORE OPERATION WERE 1 EGG, 2 EGGS, 3 EGGS, AND 4 EGGS OR MORE IN LENGTH

Length of last clutch before operation	1 egg	2 eggs	3 eggs	4 eggs or more
Average ovulation frequency	0.55	0.64	0.74	0.82
Range	(0.43–0.67)	(0.44–0.78)	(0.64–0.86)	(0.71–0.93)
Theoretical ovulation frequency*	0.50	0.67	0.75	0.80 to <1.00

* The theoretical ovulation frequency is that which would be obtained if each of these clutch lengths was preceded indefinitely by clutches of the same length, with no more than a one-day lapse between each pair of clutches.

oldest preovulatory follicle as a C_1 follicle was determined, using the percent figure derived from the equivalent normal group. This number was subtracted from the actual number of birds operated on that showed a C_1 type ovulation to give the number, in each group, that exhibited delayed ovulations. The difference between the total number of non-atretic birds, and those expected to show normal C_1 ovulations (based on the behavior of the normal birds) gave the potential number in each group of non-atretic birds which could have shown delayed ovulations, that is, the number expected to show C_2 ovulations. The actual number showing delayed ovulations divided by the number expected to show C_2 ovulations and multiplied by 100 yielded the percent delayed ovulations, or the incidence of delayed ovulations in the birds not showing atresia. B. *In the total group of birds operated on.* The number of birds showing delayed ovulations as determined above was divided by the total number in the group and multiplied by 100.

The last clutch before operation as the measure of comparison between birds. As was noted above and as will be seen in the following section, the incidence of delayed ovulations and of atresia was related to the length of the last clutch before operation. This measure of comparison was used simply for convenience in comparing the birds operated on

with the normal ones. That it is a measure, though not a direct one, of the ovulation frequency as defined and used in our previous study (Rothchild and Fraps, 1945) is seen by comparison, in Table 1, of the actual average ovulation frequency of each group of birds, classified according to the length of the last clutch before operation, and the theoretical ovulation frequency for each of these clutch lengths.

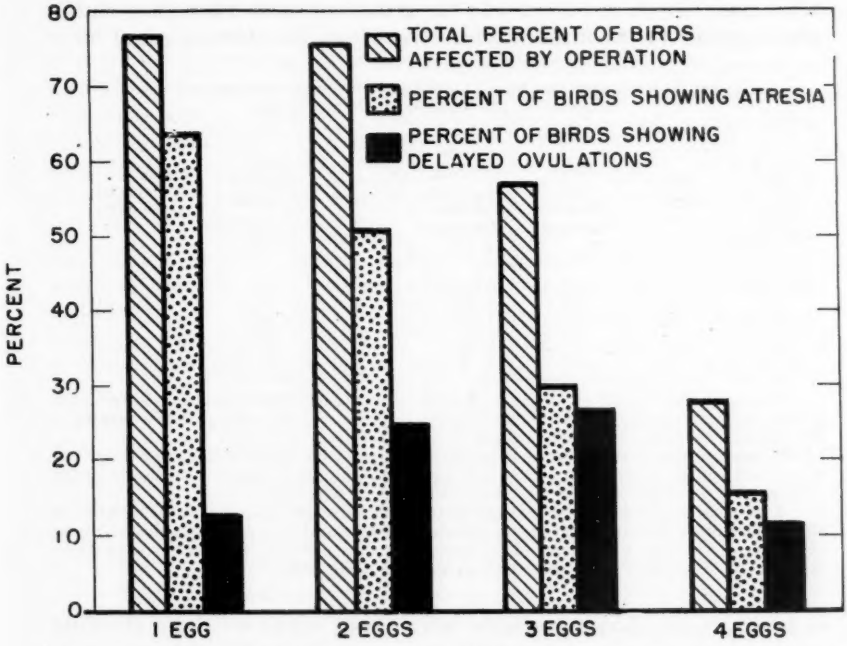

FIG. 1. The relationship between the length of the last clutch before operation and 1) the total percentage of birds affected by operation in the form of atresia and delayed ovulations; 2) the distribution of birds showing either atresia or delayed ovulations. The length of the last clutch before operation is given at the foot of each group of columns.

RESULTS

The results are recorded in Table 2 and Figure 1. Table 2 shows that the incidence of delayed ovulations among birds not undergoing atresia after operation decreased as the length of the last clutch before operation increased, in much the same way as did the incidence of atresia (Figure 1). In each group of birds with the same length of the last clutch before operation the incidence of delayed ovulations in the birds not showing atresia was equal to or greater than the incidence of atresia in the group as a whole. Comparison of the incidence of delayed ovulations in the group as a whole with the incidence of atresia (expressed graphically in Figure 1) shows that the percentage

of birds showing delayed ovulations, in general, increased relative to those showing atresia as the length of the last clutch before operation increased. Thus, approximately equal percentages of birds whose last clutch before operation was either 1 or 2 eggs showed atresia *or* delayed ovulations, but the incidence of atresia was almost 5 times as great as that of delayed ovulations in the group whose last clutch was 1 egg, while it was only slightly more than twice as great in the group whose last clutch was 2 eggs. In the groups of birds whose last clutch was 3 eggs or 4 eggs or more before operation, the percentages of birds

TABLE 2. INCIDENCE OF DELAYED OVULATIONS IN BIRDS NOT SHOWING ATRESIA AFTER ABDOMINAL OPERATIONS

Length of last clutch before operation	Birds operated on	Birds not showing atresia	Birds showing C_1 type ovulation or greater delay	Birds expected to show C_1 ovulation[a]	Birds expected to show C_s ovulation[b]	Delayed ovulations[c]	
no. eggs	no.	no.	no.	no.	no.	no.	%
1	55	20	20	13	7	7	100
2	68	33	28	11	22	17	77
3	37	26	12	2	24	10	42
4 or more	43	36	9	4	32	5	16

[a] The number of birds expected to show a C_1 type ovulation was obtained from the percentage expectations in the normal groups of birds. For preceding clutch lengths in the order given here, these percentages were, respectively, 63, 32, 9 and 11.

[b] These groups of birds, the ones in which delayed ovulations of the kind described here could appear, were obtained by subtracting the number expected to show C_1 type ovulations from the total group of birds not showing atresia.

[c] The *number* of delayed ovulations is the difference between the total number of non-atretic birds showing C_1 type ovulations and the number expected to show C_1 type ovulations. The per cent delayed ovulations is the number of delayed ovulations divided by the number of expected C_s ovulations times 100.

in both groups showing atresia were about equal to those showing delayed ovulations, but the total percentage affected in either of these two ways decreased from 56% in the group whose last clutch was 3 eggs to 28% in the group whose last clutch was 4 eggs or more.

Birds subjected to operations on the breast muscle

Thirty-seven birds were subjected to lacerations of the breast muscle. The numbers in each of the groups divided according to length of the last clutch before operation were too small for calculations of the actual incidence of delayed ovulations to have much significance, so that only the behavior of the birds in terms of total number of C_1 type ovulations, C_s ovulations and atresia will be given. In general, the results show that although the reaction to this operation was much less severe than it was to abdominal operations, the same general relationships were present. Thus, out of 9 birds whose last clutch was 1 egg, 6 showed C_1 type ovulations, 1 a C_s type ovulation, and 2 atresia. Out of 21 birds whose last clutch was 2 eggs, none showed

atresia, and only 9 a C_1 type ovulation, the remainder showing C_n ovulations. There were only 6 birds whose last clutch was 3 eggs, and only 1 whose last clutch was 4 eggs, and of these 7 birds, only 1 showed a C_1 type ovulation, the remainder showing C_n ovulations.

DISCUSSION

The data presented in the foregoing pages tend to support the idea put forth in our previous paper (Rothchild and Fraps, 1945) that the anterior pituitary is affected by the changes induced in the organism as a result of operation. The two effects of operation, atresia and delayed ovulation, are apparently related quantitatively to one another, and it is probable, therefore, that the basic cause of both effects is the same, the qualitative differential varying with the intensity of the causal agent. Although it is possible that both effects are derived from a primary affectation of the growing follicle, a more reasonable and probable assumption at present, in the light of what is known about the dependence of ovulation on the function of the anterior pituitary, together with the fact that the two effects are apparently related to one another, is that they are derived from a primary disruption of anterior pituitary function. It is interesting to note in this place that in the pigeon and ring dove delayed ovulations have been induced by injections of various steroids (Dunham and Riddle, 1942).

There are two problems which follow from this conclusion. The first is the general one of the way in which the incidence of both atresia and delayed ovulation is modified by the frequency of ovulation shown by the bird before operation. The other relates to the particular way in which a bird shows either one of these reactions.

In regard to the first problem, the relationship between the level of activity of the pituitary (which is assumed to be closely associated with ovulation frequency) and its level of resistance to the noxious effects of operation which we postulated previously (Rothchild and Fraps, 1945) we believe to be still valid. A clue to the mechanism through which this operates is afforded by a recent study by Ungar (1945). He showed that one effect of trauma in the guinea pig, the marked reduction in bleeding time, was mediated through a system in which the anterior pituitary, adrenal cortex and spleen were involved, the spleen being the organ directly responsible for production of the factor which lowered the bleeding time. It is conceivable that some such mechanism exists in the bird, so that with increasing frequencies of ovulation, mirroring increasing levels of activity of the pituitary, the level of activity of the adrenal cortex, and possibly of the spleen, increase correspondingly; when the organism is subjected to the stress of operation the counteracting agents would either be mobilized more readily, or already be present in greater quantities, the higher the bird's ovulation frequency.

The manner in which a particular response is elicited in any indi-

vidual bird is still obscure; the answer to this question is probably so closely tied up with the understanding of the basic physiological processes which give rise to the hen's ovulatory cycle that it cannot be dealt with adequately until this understanding is achieved. One starting point for an investigation of this question, however, may lie in the similarity in behavior of the normal C_1 follicle and that of the follicle showing the delay in ovulation as a result of operation. The C_1 follicle has been shown by Fraps and Dury (1942) to be ovulable with comparatively small doses of anterior pituitary preparations on the afternoon of the day on which the terminal egg of the preceding clutch is laid. Yet under natural lighting conditions the C_1 follicle never ovulates normally at this time, but early in the morning of the following day. The possibility should certainly be considered that the experimental delay in ovulation and this apparent delay in ovulation of the normal C_1 follicle are the result, in both cases, of a similar physiological status of the pituitary relative to changes in environmental factors (the light cycle for instance) or to preceding events in the ovulation cycle, or both, although the immediate causative agents may be quite dissimilar.

SUMMARY

Following abdominal operations in chickens the oldest maturing ovarian follicle either underwent atresia, or showed no effect. The incidence of both of these effects in general bore an inverse relation to the length of the last clutch before operation. In addition, the proportion of birds affected in either of these two ways changed as the length of the last clutch before operation increased. In birds whose last clutch before operation was 1 egg, 77% were affected, and the number showing atresia was about 5 times the number showing delayed ovulations. In birds whose last clutch before operation was 4 eggs or more, only 28% were affected, and the number showing atresia was only 1.33 times the number showing delayed ovulations. Birds with intermediate last clutches before operation showed results roughly intermediate between these two extremes.

REFERENCES

DUNHAM, H. H., AND O. RIDDLE: *Physiol. Zool.* 16: 383. 1942.
FRAPS, R. M., AND A. DURY: *Anat. Rec.* 84 No. 4, 3, 1942.
PHILLIPS, R. E., AND D. C. WARREN: *J. Exper. Zool.* 76: 117. 1937.
ROTHCHILD, I., AND R. M. FRAPS: *Endocrinology* 37: 415. 1945.
UNGAR, G.: *Endocrinology* 37: 329. 1945.

NATIONAL RESEARCH COUNCIL GRANTS FOR RESEARCH IN ENDOCRINOLOGY

The Committee on Research in Endocrinology, National Research Council, wishes to announce that requests for grants-in-aid during the fiscal period from July 1, 1947, to June 30, 1948, will be received until February 28, 1947. Application blanks may be obtained by addressing the Secretary, Division of Medical Sciences, National Research Council, 2101 Constitution Avenue, Washington 25, D. C. In addition to a statement of the problem and research plan or program, the Committee desires information regarding the proposed method of attack, the institutional support of the investigation and the uses to be made of the sum requested. No part of any grant may be used by the recipient institution for administrative expenses.

The Committee makes grants-in-aid of research in the general field of experimental and clinical endocrinology. However, applications for support of research in the problems of sex in the narrower sense cannot be given favorable consideration, and investigators seeking support in this field should direct their proposals to the Committee for Research in Problems of Sex of the National Research Council. The Committee on Research in Endocrinology, however, will continue to give consideration to the support of studies of the effect of sex hormones on non-sexual functions, e.g., on general metabolism and on the metabolism of steroid hormones.